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PROCEEDINGS
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# *THE THEORY OF MICRO-METEORITES.* PART I. IN AN ISOTHERMAL ATMOSPHERE*

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Communicated by Harlow Shapley, October 9, 1950

*Introduction.*—In 1946, during the great Giacobinid meteor shower, H. E. Landsberg[1] collected several small magnetic particles that apparently were associated with the shower. Since some of these particles, a few microns in length, were extremely angular in shape (wedge-shaped and opaque), it seemed unlikely that they could have been the end-products of vaporizing meteors. Landsberg concluded that they must have been stopped by the atmosphere *without being heated above their melting-points*. As a result of his suggestion I have developed the present theory to investigate the process whereby temperature radiation can dissipate the energy gained by encounters with atmospheric molecules sufficiently rapidly to permit finite meteoric particles to be stopped without melting. Some basic concepts of this theory have been discussed by E. Öpik[2] and an application made in the case of an isothermal atmosphere.

The term *micro-meteorite* appears to be an appropriate designation for one of these small particles.

In every sense the micro-meteorites represent the lower extreme to the ascending sequence embracing *meteor*, *fireball* and *meteoritic crater formation*. Hence, the theory is a limited meteor theory, partially applicable to the unobservable beginning of a meteor.

We may now assume and later (Part II) prove that interaction between the air molecules striking and those leaving the micro-meteorite may be neglected. The molecular mean free paths, even after correction for the relatively slow velocity of air molecules thermally emitted, are greater than the linear dimensions of the micro-meteorite.

Let us suppose that the micro-meteorite presents a certain surface area, $A$, to the atmosphere, which it encounters with velocity, $V$. This

surface may be an average frontal area in case the body is rotating, or a fixed area in case the body does not rotate. If $A(\phi, \theta)$, a function of the ordinary spherical coordinates, represents the actual cross-sectional profile of the micro-meteorite as seen from direction $\phi$ and $\theta$, then the average frontal area, $A_1$, is given by

$$A_1 = \frac{1}{4\pi} \int_{\phi=0}^{\pi} \int_{\theta=0}^{2\pi} A(\phi, \theta) \sin \theta \, d\phi \, d\theta. \qquad (1)$$

This average area, $A_1$, may or may not equal the frontal area $A$. The thermal radiating area, $B$, however, will be specific for a particle of a given shape; it is given by

$$B = 4A_1. \qquad (2)$$

Let us assume that the temperature of the micro-meteorite is at all times uniform over the area $B$. This assumption is equivalent to an assumption that the heat conductivity is infinite or that the heat capacity is zero. We shall first develop the theory on the assumption that the heat capacity is negligible and later investigate the nature of the error made.

The air molecules impinge on the forward surface with relative velocity, $V$, because, by definition, the mean free path of the outgoing molecules relatively to the moving body is larger than its linear dimensions. To evaluate the energy transfer to the surface of the body we may make use of the concept of the accommodation coefficient, $\alpha$, which is defined in terms of the kinetic energy of the air molecules, referred to the coordinate system of the moving body. The accommodation coefficient is, then, the actual loss of kinetic energy by the air molecules, as a result of the encounter, divided by the loss if all of them were momentarily to adhere to the surface and be re-emitted at the thermal velocity corresponding to the surface temperature. Since the thermal energies, both original and at the surface temperature, are relatively small compared to the kinetic energy at velocity, $V$, these energies may be neglected with an error less than 1% (minimum $V$ = 11.2 km./sec.). Hence $\alpha$ represents the fraction of the molecular energy at velocity, $V$, that is transmitted to the micro-meteorite.

Since the air molecules will encounter the body with relative energies of the order of 8 to 800 electron volts, while the work function of the surface will be only a few volts, the molecules will certainly penetrate the surface for several molecular layers except at the lowest velocities. We must conclude that few of them will leave with high velocities; the losses by dissociation, excitation and ionization can be only a few volts. Hence $\alpha$ must be nearly unity at most velocities.

In air of density, $\rho$, the micro-meteorite will meet in time, $dt$, an air mass, $dm_a$, given by

$$dm_a = A\rho V\,dt. \tag{3}$$

The corresponding energy gain, $dE_g$, amounts to

$$dE_g = \frac{\alpha}{2} V^2\,dm_a = \frac{\alpha}{2} A\rho V^3\,dt. \tag{4}$$

Part of this energy will be utilized in raising the temperature of the meteoroid, part radiated by black-(or gray)-body radiation, part used in dissociation, excitation and ionization and perhaps part used in disengaging material from the surface. If the accommodation coefficient is defined to include the dissociation, excitation and ionization and if vaporization is negligible, we may deal here explicitly with only the heating and radiation terms.

We may assume that the meteoroid was previously in temperature equilibrium with the night side of the Earth (or Sun and Earth during the day) at temperature $T_0$. With a gray-body emissivity coefficient of $\beta$, the loss of energy by radiation, $dE_r$, of a surface at temperature $T_s$ is

$$dE_r = \beta B\sigma\,(T_s^4 - T_0^4)dt, \tag{5}$$

where $\sigma$ is the Stefan-Boltzmann constant.

If the meteoroid is small ($s \ll 1$ cm.), of mass, $m$, and if the coefficient of heat conductivity is at all comparable to that of ordinary rocks, the internal temperature should differ negligibly from the surface temperature in time intervals somewhat smaller than one second. The permissible limits to this assumption will be discussed later. If, then, the heat capacity per gram is $C_s$, the temperature will vary as

$$mC_s dT_s = dE_g - dE_r. \tag{6}$$

By equations (4) and (5), equation (6) becomes

$$mC_s \frac{dT_s}{dt} = \frac{\alpha}{2} A\rho V^3 - \beta B\sigma(T_s^4 - T_0^4). \tag{7}$$

The precise conditions under which the heat capacity in the left member of equation (7) can be neglected are not apparent *a priori*. We can, however, easily determine a resultant rough limit to the dimensions of the micro-meteorite and later study the question more thoroughly. The maximum temperature, $T_m$, to which a micro-meteorite can be heated without appreciable vaporization is just below the melting point of the least refractory material in the meteoroid, approximately 1200°K. to 1700°K. for typical stones.[3] Iron, iron oxides and silica also fall within this range. The temperature rise from the equilibrium temperature at the Earth to $T_m$ is relatively large. Generally this rise will occur over a considerable distance through the atmosphere since the radiation varies as $T_s^4$ while the

heating is proportional to atmospheric density. We will find that the heat capacity is negligible for fast micro-meteorites because they are so small. For slow micro-meteorites an atmospheric density increase of a factor of two will require roughly a half-second of atmospheric traverse at normal incidence.

Hence, if the heat radiation in an interval of the order of half-second near maximum temperature is large compared to the heat required to raise the temperature from $T_0$ to $T_m$ we may safely neglect the heat capacity in equation (7). The condition just defined is

$$2mC_s(T_m - T_0) \ll \beta B\sigma (T_m^4 - T_0^4). \tag{8}$$

The limiting radius, $s$, for a spherical meteor of density, $\rho_s$, is, from equation (8)

$$s \ll \frac{3\beta\sigma}{2C_s\rho_s}(T_m^3 + T_m^2T_0 + T_mT_0^2 + T_0^3). \tag{9}$$

The right member of equation (9) is of the order of 0.01 cm. for an iron or stony meteorite. Hence we may safely ignore the heat capacity of micro-meteorites of radii less than 10 microns.

With our current assumptions then, we may set the left-hand member of equation (7) equal to zero and determine the surface temperature of a micro-meteorite as a function of its velocity and the atmospheric density. The result is

$$T_s^4 - T_0^4 = \frac{\alpha A\rho V^3}{2\beta B\sigma}. \tag{10}$$

While the temperature of the micro-meteorite is rising, its velocity is being reduced by atmospheric resistance. We may define the drag coefficient, $D$, by the equation

$$mdV = -\frac{ADpV^2}{2}dt. \tag{11}$$

The drag coefficient will include a major component, of the order of 2 numerically, if we assume that air particles momentarily adhere to the meteoroid and are reemitted isotropically. A smaller additional term will arise from the non-isotropic reemission according to the hypothesis of Tsien[4] and Miss Heineman.[5] No term will arise from evaporation of material, however, since we assume (perhaps erroneously at high velocities) that evaporation is negligible.

A rigorous inclusion of the reemission drag term would involve the surface temperature of the micro-meteorite. Since the effect is rather small, we may include it approximately after a solution has been made with $D$ constant.

The simultaneous solution of equations (10) and (11) will provide a relation among the temperature, velocity and time, if the atmospheric density can be expressed as a function of the time. A complicated, variable and poorly known relation between atmospheric density and height actually exists at the atmospheric heights concerned. In the following sections the fundamental equations will be solved for certain types of such relations.

*The Solution in an Isothermal Atmosphere.*—Let us assume that the atmosphere is isothermal and that the mean molecular weight of the air is also constant. Then the atmospheric density, $\rho$, varies with the height, $h$, above sea level according to the relation

$$\rho = \rho_0 e^{-bh}, \tag{12}$$

where $b$, the logarithmic density gradient, is a positive constant and $\rho_0$ is constant.

Let us neglect the curvature of the Earth. Then for a particle entering the atmosphere with velocity, $V$, from an apparent radiant at zenith distance, $Z$, the time and height are related by the equation

$$dh = -V \cos Z \, dt. \tag{13}$$

Since meteoric velocities are so large we may neglect the effect of the Earth's gravity, assuming that the pertinent part of the trajectory is a straight line, and that the velocity is unaffected by gravity.[6]

Equations (11), (12) and (13) then lead to the following differential relation between velocity and height:

$$\frac{dV}{V} = \frac{AD\rho_0}{2m \cos Z} e^{-bh} \, dh. \tag{14}$$

We have already assumed that all of the quantities in equation (14) except $V$ and $h$ may be treated as constants. Hence we may integrate equation (14) between the limits of $V_\infty$, the initial velocity at great heights essentially infinity, to any pertinent velocity, $V$, and height, $h$. The integral of equation (14), when combined with equation (12) to eliminate the height explicitly, yields the following relation between the velocity and atmospheric density:

$$\log (V/V_\infty) = -\frac{AD\rho}{2bm \cos Z}. \tag{15}$$

Now the micro-meteorite can traverse the atmosphere undamaged only if the velocity is reduced sufficiently to prevent the surface temperature from exceeding a critical temperature, $T_m$, somewhat below the melting point of the meteoritic materials. This restriction can be imposed if we eliminate the density, $\rho$, between Equations (15) and (10) and then impose a maximal condition on $T_s$. The first step gives

$$T_c^4 - T_0^4 = -\frac{\alpha b m \cos Z}{\beta \sigma B D} V^3 \log (V/V_\infty). \qquad (16)$$

To determine the maximum temperature from equation (16) we must make assumptions as to the functional forms of the accommodation coefficient, $\alpha$, and the emissivity factor, $\beta$. In view of our lack of knowledge of the detailed physical and chemical structure of the micro-meteorites and, even with this knowledge, the uncertainty in the physical laws for $\alpha$ and $\beta$, we may as well adopt constant values for these quantities as well as for $D$. Hence, from the derivative of equation (16) the maximum temperature is reached at the critical velocity, $V_c$, given by

$$\log (V_c/V_\infty) = -\frac{1}{3}, \qquad (17a)$$

or

$$V_c = V_\infty/e^{1/3} = 0.7165\ V_\infty, \qquad (17b)$$

where $e$ is the base of the natural logarithms.

We may now solve for the ratio of the mass to the effective surface area, $m/B$, of the micro-meteorite from equation (16) for the maximum temperature, $T_m$. The resulting critical ratio is

$$m/B = \frac{3e\beta\sigma D(T_m^4 - T_0^4)}{\alpha b \cos Z V_\infty^3}. \qquad (18a)$$

Equation (18a) represents the maximum value of $m/B$ for a micro-meteorite that is to be stopped, undamaged by the atmosphere. The maximum radius for a spherical particle of radius, $s$ (sphere) and density, $\rho_s$, is then

$$s \text{ (sphere)} = \frac{9e\beta\sigma D(T_m^4 - T_0^4)}{\alpha \rho_s b \cos Z V_\infty^3}, \qquad (18b)$$

a result corresponding closely to Öpik's Equation (53).[2]

It is of interest to note from equations (18) that the maximum particle dimension depends directly upon the critical temperature to the fourth power and upon the inverse cube of the original velocity. Low velocity and high melting point strongly favor the passage of such a particle through the atmosphere. Irregular or elongated shape favors the process for a given meteoric mass. Because of the cos $Z$ term, larger masses may enter at lower angles of incidence.

For a rapidly spinning micro-meteorite in the shape of a right circular cylinder of length $l$ and radius $s$ (cyl.), the maximum radius in terms of the comparable sphere (equation 18b) of the same density and $m/B$ ratio is given by

$$s\ (\text{cyl.}) = s\ (\text{sphere}) \times \frac{2}{3}\left(\frac{s+l}{l}\right)\ (\text{cyl.}). \qquad (18c)$$

If the rapidly rotating cylindrical meteorite is to have the same radius as the spherical one, then the length must be equal to the diameter of the sphere. An extremely long cylindrical meteorite ($l \gg s$) will be stopped when its radius is 2/3 or less the corresponding spherical radius (equation 18b). The maximum permissible dimensions of micro-meteorites with other shapes or orientations can be determined by means of equations (1), (2) and (18).

In applying equations (18) approximately to an atmosphere of variable temperature it is necessary to obtain an appropriate value of the logarithmic density gradient, $b$. The critical altitude is at the point of maximum temperature for the micro-meteorite. From equations (15) and (17) we find the critical density, $\rho_m$, at maximum temperature $T_m$ as follows:

$$\rho_m = \frac{2bm \cos Z}{3AD}. \qquad (19)$$

If the frontal area of the meteorite, $A$, is approximated by the average area, $A_1$, equations (2) and (18a) transform equation (19) into

$$\rho_m = \frac{8e\beta\sigma(T_m^4 - T_0^4)}{\alpha V_\infty^3}. \qquad (20)$$

The value of $b$ and the height corresponding to $\rho_m$ may be derived from some standard atmosphere. The fact that the shape and mass of the micro-meteorite and cos $Z$ do not enter equation (20) is rather surprising. In case there is reason to believe that the actual frontal area differs from the average cross-sectional area, a correction term can easily be applied for this factor in equation (20).

The drag coefficient, $D$, is a vital factor in equations (18) and (19). In case the air molecules impinging on the surface of the micro-meteorite are momentarily "captured" by the surface, but very quickly reemitted with energies corresponding to the temperature of the surface, $D$ is equal to 2 with a small correction term for the Tsien-Heineman effect mentioned above. This situation corresponds to that when the accommodation coefficient, $\alpha$, is exactly unity. M. L. Wiedman[7] finds that the accommodation coefficient for air on various metals is generally close to 0.9. At the higher energies here considered, there is little doubt that the air molecules will largely penetrate the surface molecular layers and be momentarily captured. Since only a few can combine chemically and since condensation is impossible at the temperatures considered, there appears to be little question that essentially all of the air molecules will be captured and then reemitted with an average velocity, $v_T$, corresponding to the temperature of the surface. The average velocity is given by

$$(v_T)^2 = \frac{8k_1T_s}{\pi\mu_1}, \tag{21}$$

where $k_1$ is the gas constant and $\mu_1$ is the molecular weight of the gas.

If the micro-meteorite is assumed to be spherical and the reemission is uniform with respect to solid angle from a small area of the surface, the momentum transfer to a hemisphere per average molecule is $4\mu v_T/9$. Since the time lag of reemission must be small compared to a possible rotation period of the micro-meteorite, this momentum transfer will oppose the motion of the body. If we further accept $D = 2$ as the best approximation to the drag coefficient without including reemission, the value of $D$ becomes:

$$D = 2[1 + 4v_T/(9V)]. \tag{22}$$

A numerical calculation shows that the $v_T$-correction in equation (22) amounts at most to a few per cent in the velocity and temperature ranges under consideration. Most of the deceleration of interest occurs fairly near the critical region of maximum temperature. In adopting a constant value of $D$, therefore, we may use $v_T$ applying at the maximum temperature and compensate this error somewhat by adopting $V = V_\infty$ in equation (22).

It would be desirable to make some correction in the drag coefficient for the fact that some of the impinging air molecules must deviate from the assumed process of penetration and thermal reemission, but we have no detailed information as to the surface characteristics of micro-meteorites. The relative error made in $D$ by this omission is less than $1 - \alpha$.

The remaining undetermined constant in Equation (18) is the emissivity factor, $\beta$. Nothing precise can be known about this factor until micro-meteorites have been studied carefully in the laboratory. Probably $\beta$ is very near unity. For want of better information we may set $\alpha = \beta \cong 1$, so that their effects cancel in equation (18). We may adopt $T_m = 1600°\text{K.}$, and $T_0 = 300°\text{K}$. The remaining quantities to be specified in equation (18a) concern the atmosphere. The Tentative Standard Atmosphere of the National Advisory Committee for Aeronautics[4] represents good modern estimates of upper-atmospheric densities and will be used for the present calculation.

For a velocity of 23 km./sec., corresponding to the 1947 Giacobinid Meteor Shower, the atmospheric density at which a micro-meteorite of limiting dimensions attains maximum temperature ($\rho = 8.2 \times 10^{-10}$ gm./cm.$^3$) occurs near the 112-km. level, by application of equation (20). With an air temperature of 346°K. and $b = 1.1 \times 10^{-6}$ cm.$^{-1}$ the maximum radius of spherical iron particles for $\cos Z = 0.45$ are then calculated to be about 4 microns. Long cylindrical particles might have a diameter as great as 6 microns, in good agreement with Landsberg's observations mentioned earlier.

Part II of this paper will concern the solution of limiting dimensions for micro-meteorites in an atmosphere of constant temperature gradient and in the general case. A critical discussion of the assumptions adopted here will be included as well as some elaboration of the numerical results.

* This paper was written as a part of the investigation for United States Naval Ordnance Contract-10449-05512.

[1] *Pop. Ast.*, **55**, 322 (1947).

[2] *Pub. Univ. Tartu*, **29**, No. 5, 51 (1937).

[3] See Daly, R. A., *Igneous Rocks and the Depths of the Earth*, McGraw-Hill Book Co., 1933, p. 65.

[4] Tsien, Hsue-Shen, *J. Aero. Sci.*, **13**, 653 (1946).

[5] Heineman, M., *Comm. Apl. Math.*, **1**, 259 (1948).

[6] See, e. g., Whipple, F. L., *Proc. Am. Phil. Soc.*, **79**, 499 (1938).

[7] See Tsien, *loc. cit.*

[8] Warfield, C. N., NACA, Tech. Note, No. 1200, Langley Field, Jan., 1947.

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## *MERCAPTAN-INDUCED COAGULATION OF PROTEINS**

BY CHARLES HUGGINS, DONALD FRASER TAPLEY AND ELWOOD V. JENSEN

FROM THE NATHAN GOLDBLATT MEMORIAL HOSPITAL FOR NEOPLASTIC DISEASES OF THE UNIVERSITY OF CHICAGO, CHICAGO, ILL.

Communicated, October 29, 1950

In this paper it will be demonstrated that mercaptans possess the property of inducing coagulation of certain proteins at room temperature and neutrality. This effect throws light on the importance of —S—S— bonds in the intramolecular folding of several proteins in the native state.

While the dispersive action of mercaptans on keratin is well known, the coagulative action on soluble proteins apparently has not been described. Goddard and Michaelis[1] found that wool readily dissolves at room temperature in thioglycollate solutions at pH 10–13 because of reduction of disulfide bonds. The following reaction[1–4] occurs in the thiol-disulfide system:

$$RS—SR + 2R'SH \rightleftarrows 2RSH + R'S—SR'$$

Since greater alkalinity was required in the experiments of Goddard and Michaelis than was needed for the reduction of cystine by thiols, they[1] postulated that an additional intramolecular bridge must be broken before disulfides of keratin can be reduced; they assumed that the second bridge was salt-like in character and that it was disrupted by removal of a proton from the amino group in alkaline solution. Jones and Mecham[5] observed that a variety of keratins were dispersed at pH 7 and 40°C., by adding urea or anionic detergents to the mercaptan solution. Mirsky and Anson[2] found that thioglycollate in excess completely reduces disulfide groups in

denatured plasma albumin and egg albumin; in contrast sodium sulfite reduces only one-half of the cystine present.

*Method.*—Crystalline bovine plasma albumin and egg albumin and refined bovine γ-globulin (95 per cent homogeneous on electrophoresis) were studied. Acetylated bovine albumin was prepared by the method of Hughes as described by Olcott and Fraenkel-Conrat.[6] Analysis indicated that 78 per cent of the free amino groups were acetylated.

Unless otherwise stated the proteins were dissolved in 0.1 *M* phosphate buffer, pH 7.4, and the experiments were carried out in 13 × 100 mm.

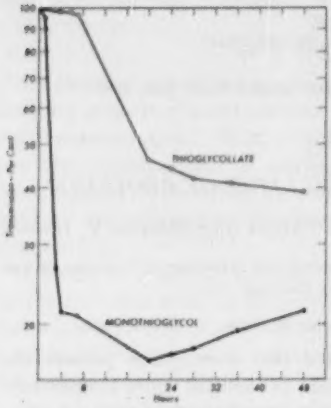


FIGURE 1

Rates of development of turbidity in plasma albumin induced by 0.2 *M* solutions of monothioglycol, and sodium thioglycollate. In each case the system consisted of bovine plasma albumin 50 mg., mercaptan compound 2 m. eq. and 0.1 *M* phosphate buffer, pH 7.4, to make the final volume 10 ml.; temperature 37°.

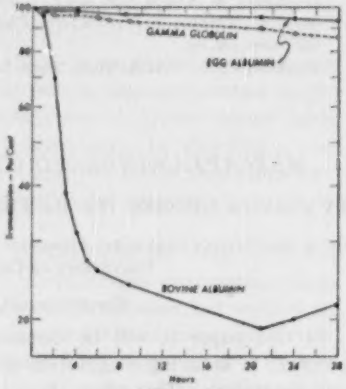


FIGURE 2

Differential rates of development of turbidity in various refined protein fractions induced by 0.2 *M* mercaptoethanol. The system consisted in each case of 50 mg. protein, mercaptoethanol 2 m. eq., and 0.1 *M* phosphate buffer pH 7.4 to make the final volume 10 ml.; temperature 37°.

glass tubes at 37°. Turbidity was determined in a Coleman spectrophotometer at λ = 660 millimicrons, using 19- × 150-mm. tubes. A protein solution was considered to be coagulated[7] when it failed to pass through a clean dry stainless steel screen (8 mesh, wire diameter 0.7 mm.).

*Experimental.*—When a mercaptan is added to a solution of bovine plasma albumin turbidity soon develops and increases to a maximum (Fig. 1), then decreasing slightly. The viscosity of the solution increases until a firm opaque gel forms. Egg albumin and γ-globulin behave in a similar fashion except that the reaction is much slower (Fig. 2). Dialysis

for 5 days against water produces no apparent change in these white mercaptan-induced gels.

An inverse relationship exists between the protein concentration and the minimal quantity of thioglycollate required to form a gel; at pH 7.4 and 37° the thioglycollate requirement decreased progressively from a concentration of 0.34 *M* to 0.08 *M* as the albumin concentration was increased from 1.2 to 5 per cent. At 5°, however, neither turbidity nor gels developed in 5 per cent albumin even after 72 hr. in 0.4 *M* thioglycollate.

In solutions of 7 per cent albumin, turbidity with subsequent gelation developed with the following agents: mercaptoethanol (monothioglycol), sodium thioglycollate, reduced glutathione, cysteine and sodium hydrosulfite. Mercaptoethanol produced turbidity more rapidly (Fig. 1) and gel formation at a lower concentration than did the other reducing agents employed. No gels or turbidity occurred in 7 per cent albumin solutions with the following reagents in concentration up to 0.4 *M*: sodium cyanide, potassium ferrocyanide, hydroquinone or sodium sulfite. Slight turbidity but no gel formation occurred in 0.4 *M* sodium ascorbate.

Coagulation of albumin by thiol compounds is favored by an increase in pH of the mixture. With increasing pH the minimum concentration of sodium thioglycollate required to form a gel in 2.5 per cent solutions of plasma albumin decreased progressively from 0.18 *M* at pH 7.0 to 0.06 *M* at pH 8.4. No monophasic gels were observed in albumin solutions treated with thioglycollic acid at pH values below 6.8. Between pH 5 and 6.6 the protein solution remained clear in 0.2 *M* thioglycollic acid while at pH 4.5 a synerizing precipitate formed.

Variation of the salt concentration over the range 0.1 to 1.1 *M* had no apparent effect on mercaptan-induced gelation of albumin. At salt concentrations less than 0.02 *M* gel formation was markedly inhibited, while at salt concentrations of 2–3 *M* the coagula formed were quite friable. Oxygen apparently plays no part in the mercaptan-induced coagulation of albumin; solutions degassed by repeated freezing and thawing *in vacuo* and sealed in the limbs of A-tubes evacuated to $10^{-2}$ mm. of Hg before mixing behaved identically with control mixtures exposed to air.

Partial acetylation (78 per cent) of the amino groups of plasma albumin resulted in a twofold increase in the mercaptan concentration required to form a gel; in a 2.5 per cent protein solution at pH 7.4, 0.27 *M* thioglycollate was required instead of 0.15 *M* as with unmodified albumin. The gels thus formed from acetylated albumin were markedly less turbid than those formed from unmodified albumin itself.

The opaque gels formed by the mercaptans are rapidly dissolved by the addition of urea in final concentration of 5 *M* or greater with nearly complete disappearance of the turbidity. Now urea itself possesses the ability to induce gelation of albumins and certain other proteins.[8, 9] These

coagula are quite different from mercaptan induced gels, being translucent and very cohesive. The protein gels induced by urea are dissolved rapidly by the addition of mercaptans.[10]

*Discussion.*—Two types of chemical bonds have been established as forces which maintain the protein molecules in specific configuration, namely, hydrogen bonds and disulfide bridges. In the theory of Mirsky and Pauling[11] the polypeptide chain of a native protein is folded and retained in its unique configuration by hydrogen bonding between the peptide nitrogen and oxygen atoms and also between the free amino and carboxyl groups of the diamino and dicarboxylic amino acid residues. Other workers have postulated that cystine[12] must form one of the side links between adjacent polypeptide chains in the protein molecule.

Classes of compounds which disrupt either type of linkage lead to the denaturation of a protein such as plasma albumin. Amides are well-known hydrogen-bond-forming substances which can rupture the hydrogen bonds involved in maintaining the protein in the native state; mercaptans reduce —S—S— linkages. With each type of reagent the denatured molecules aggregate, the gels varying in properties because of differences in the denatured protein molecule.

The coagulation of albumin by urea has recently been interpreted[10] as follows: Concentrated amides by competition with intramolecular hydrogen bonds of the native protein disrupt its structure and make disulfide groups reactive to small concentrations of sulfhydryl. By means of a chain reaction between protein —SH and —S—S— groups a reticulum is then knitted together consisting of intermolecular protein disulfide bonds which hold together the unfolded protein chains. Solvent is bound to the protein within this framework so that clear firm gels result.

Mercaptans appear to coagulate certain soluble proteins by a different mechanism, namely, disruption of the protein configuration through the cleavage of disulfide bonds followed by aggregation of the denatured protein through hydrogen bonds. Plasma albumin undergoes these changes more rapidly than $\gamma$-globulin or egg albumin implying that the disulfide groups of albumin are more readily available to reduction of —SH groups than in the other proteins.

The ready dispersion of the opaque mercaptan-induced gels upon the addition of urea indicates that aggregation in these gels is chiefly through intermolecular hydrogen bonding. Further evidence is derived from the deficient capacity of acetylated albumin to coagulate in the presence of mercaptans, due to a decrease in the number of potential hydrogen bond formers, the amino groups. The absence of any oxygen effect in mercaptan-induced gelation makes it doubtful that reoxidation of reduced disulfide bonds plays a major part in the aggregation.

*Summary.*—Mercaptans and hydrosulfite denature and subsequently

coagulate bovine plasma albumin, γ-globulin and egg albumin to form opaque gels at room temperature and neutrality. The reaction with bovine plasma albumin is many times more rapid than with the other two proteins. Gels so formed rapidly dissolve in concentrated solutions of amides such as urea. The capacity of albumin to form this type of gel is decreased by partial acetylation of the albumin. The evidence indicates that mercaptan-induced gelation occurs through hydrogen bonding between the extended denatured protein molecules.

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## *THE ACTION SPECTRUM OF THE INHIBITION OF GALACTOZYMASE PRODUCTION BY ULTRAVIOLET LIGHT**

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Certain yeasts, when placed in contact with galactose, acquire the ability to use that substrate after a period of time. Such a response is called enzymatic adaptation and will take place even in the absence of a nitrogen source. In recent years, with the realization that at least one of the components of any enzyme is a protein, it has been recognized that in adaptive enzyme formation we have a case where specific protein material is synthesized by resting cells.

It has been demonstrated that ultra-violet radiations from a sterilamp inhibit the aerobic adaptation of yeast to galactose.[1] Determination of an action spectrum for this effect might enable us to identify the cellular constituent affected by the radiation. The problem is of theoretical in-

terest because a clue might be found to the mechanism of adaptive enzyme synthesis. An action spectrum has been determined and is reported herein.

The theoretical basis and the limitations of the action spectrum method are discussed by Loofbourow.[2] The reciprocals of the energy required to produce a given effect when plotted against the wave-length give a curve which is called the action spectrum. This is thought to correspond to the absorption spectrum of some photolabile compound responsible for the biological effect being considered; the absorption spectra of known compounds are matched with the action spectrum. A summary of ultra-violet action spectra is given by Giese.[3]

*Experimental.*—In the present experiments the same strain of yeast was used as in the previous experiments.[1] Cultures were grown at 28°C. for 24 hours in liquid medium (30 ml. 3% dextrose, 1% yeast extract in 250 ml. flasks) on a mechanical shaker. The cells, washed as before, were suspended for experiments in M/20 $KH_2PO_4$ in a concentration of $2 \times 10^8$ cells per ml.

TABLE 1

MINIMAL DOSES REQUIRED TO PREVENT GALACTOZYMASE FORMATION FOR AT LEAST FOUR HOURS

| WAVE-LENGTH, Å. | DOSE, ERGS/MM.$^2$ |
|---|---|
| 2345 | 3374 |
| 2537 | 1687 |
| 2654 | 1687 |
| 2804 | 3374 |
| 3025 | 13,596 |
| 3130 | >54,384 |

Ultra-violet light obtained from a quartz mercury arc was passed through a quartz monochromator, and the intensity of the light was measured with a thermopile galvanometer system as described elsewhere.[4] The monochromatic light struck the suspension of cells from below. The volume of cells irradiated was 0.06 ml. Stirring during irradiation was performed by a moving jet of compressed air saturated with water vapor. Precautions were taken against evaporation; if evaporation was excessive, the sample was discarded. Once irradiation had begun, the sample was handled only in red or yellow light to prevent photoreactivation.

Following irradiation, the sample was transferred to the reaction cup of a capillary microrespirometer of a modified Kirk-Stern type,[5] developed by the author and Mr. Robert Perthel of the Stanford Research Institute. A 0.06-ml. sample of 4% galactose solution was added to the suspension with a braking pipette, and the course of the respiration followed by recording the progress of a kerosene drop in the capillary tube. Thermal regulation of the system was accomplished by submerging the cups at either end of the U-shaped capillary in a vessel of water at room tempera-

ture. Stirring the yeast in the reaction cup to keep it well aerated was performed by means of a tiny magnetic flea made of a piece of fine steel wire sealed in a glass capillary.

The inhibiting effects of six different wave-lengths were studied: 2345, 2654, 2804, 3025 and 3130 Å. For comparison of efficiency, determinations were made of the minimum doses required to prevent adaptation for four hours after addition of galactose. Normal adaptation time of this strain of yeast is 90 minutes.[1] The criterion of adaptation was a measurable increase in the rate of respiration above the endogenous before 240 minutes had elapsed. In doubtful cases the experiment was followed for another hour. Table 1 shows the results which were obtained, each being the result of at least three series of experiments.

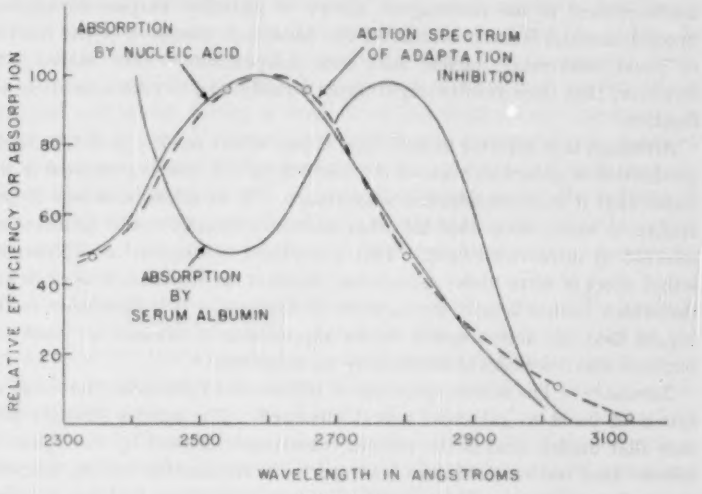


FIGURE 1

Action spectrum for inhibition by ultra-violet light of adaptation of yeast to galactose.

Analysis of the above data reveals that there is maximum efficiency in the region of 2600 Å. with a decrease on either side. If the relative efficiency is plotted against wave-length so that the maximum of the action spectrum is at the same height as that of the absorption spectrum of nucleic acid, it is seen that there is excellent agreement between the two.

*Discussion.*—We have seen in Fig. 1 that the absorption spectra of unconjugated proteins such as serum albumin differ markedly from that of nucleic acid. Unconjugated proteins, due to the aromatic amino acids they contain, have an absorption peak in the region of 2800 Å.; nucleic acids absorb maximally at 2600 Å. because of their purine and pyrimidine

rings. On the basis of present evidence and theory, it would have been reasonable to expect that we would obtain an action spectrum of either, since both have been implicated in protein synthesis. The subject of synthesis of proteins, including adaptive enzymes has been reviewed by Northrop.[6] He points out that enzymes, themselves proteins, must catalyze the formation of other proteins. In addition, Northrop advances the hypothesis that protein synthesis occurs in two steps. The first is the auto-catalytic formation of proteinogens or "type" proteins. The second step is the production of stable proteins by either catalytic or non-catalytic reactions. The first requires energy, the second does not. The proteinogen, though hypothetical, is thought to contain nucleic acid because of its autocatalytic formation. Spiegelman also assigns a prominent role to nucleoprotein in his plasmagene theory of adaptive enzyme formation.[7] Spiegelman and Kamen correlated the phosphate turnover of the fraction of yeast containing nucleic acid with adaptation.[8] They stated later however, that their results might apply equally well to constituents in the fraction.[9]

Although it is difficult at this time to say where nucleic acid acts in the production of galactozymase, it is evident from the results presented in this paper that it is of considerable importance. If we allow ourselves to generalize, it would seem that all other protein synthesis would be adversely affected by ultra-violet light. This is not hard to imagine, considering the lethal effect of ultra-violet radiations; death is the logical fate of an organism which cannot replace its outworn protoplasm. It is significant in this regard that the action spectrum for suppression of division of yeast and bacteria also resembles absorption by nucleoprotein.[3, 10, 11]

*Summary.*—The action spectrum of ultra-violet light inhibition of adaptation of yeast to galactose was determined. The results strongly indicate that nucleic acid is the cellular constituent affected by the light. It follows that nucleic acid is at least partially responsible for the adaptive enzyme production. The possible significance of these findings is briefly discussed.

* Part of a thesis submitted as a partial requirement for the Ph.D. The author is indebted to Dr. Arthur C. Giese who suggested the problem and helped in its execution.

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## *THE STRUCTURE OF THE HETEROCHROMATIC PART OF THE Y-CHROMOSOME IN DROSOPHILA BUSCKI*[*]

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In the salivary glands of *D. buscki* the *Y*-chromosome was at first shown to be a small euchromatic element whose length is about $^1/_{34}$ of the *X*-chromosome, and consisting of some 14–15 bands.[1, 2] In ganglial metaphases, this *Y*-chromosome has the form of a tapering rod, narrow at the proximal end which carries a small knob-like thickening; the length is about equal to the *X*-chromosome. This non-correspondence between the appearance of the *Y*-chromosome in the two types of cells was attributed to a heterochromatic or inert part, which is greatly reduced in relation to length, or apparently absent, in salivary glands. Until recently few attempts were made to identify such heterochromatic elements in salivary gland cells.

It has been shown previously that the *Y*-chromosome in *D. buscki* is necessary for the life of males: *XO*-males die at an early stage of the development.[3–5] As a result of a comprehensive cytogenetic investigation on the *Y*-chromosome in *D. buscki*, it has been possible to clarify the role of constituent parts in some detail, and to show their action on the development of males. In particular, it has been shown that the *Y*-chromosome is two-armed: the right, short arm, is the knob-like thickening at the proximal end, and is the euchromatic element in the salivaries; the left, long arm, is the inert heterochromatic part (unpublished).

New information concerning the structure of this inert part has also been obtained in the course of this work, and is reported in the present paper.

*Experimental Data.*—The material for this work was the mutant line *Curled-Y(404)* obtained in an $F_1$ from crossing X-rayed males to wild type females. The ends of the wings of the mutant flies are bent upwards. This mutant behaves genetically as Y-linked, that is, it is inherited from father to son. Among the offspring of mutant males, about 10–12% of non-mutant males appear. This last class of males have a slightly shortened body, and are completely sterile.

Cytological analysis of the mutant males shows a break in region 7 of IIR. The proximal part of the broken IIR usually pairs fully with its

unbroken partner, while the distal part usually pairs only partially, or lies entirely free in the nucleus near the proximal ends of the other chromosomes. In the class of sterile males mentioned above (second class) a trivalent for the distal part of IIR is seen. This third, short element, which is the distal part of IIR seen in mutant males, sometimes pairs with its normal partners, or sometimes is free. The euchromatic *Y*-chromosome element in either type of male is not involved in the abberation. In ganglial metaphases of mutant males there is one autosome with a markedly longer arm and a *Y*-chromosome which is short and two-armed. The males of the second class have normal autosomes, but also a short two-armed *Y*-chromosome (these males are hypoploid for a part of the *Y* and hyperploid for the distal part of IIR).

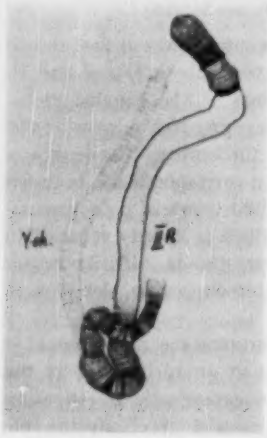


FIGURE 1

Translocation involving the left arm of the *Y* and the right arm (IIR) of the second chromosome in *D. buscki*.

From all these data, it is apparent that we are here dealing with a reciprocal translocation between the *Y* and IIR. The position of the break in IIR is known; in the *Y*-chromosome, the break is in the proximal part, near its knob-like end. One arm of the new *Y*-chromosome is this knob-like thickening, the other arm is the distal part of the translocation IIR. The long heterochromatic arm of the *Y*-chromosome is translocated to the top of the proximal part of IIR, and the lengthened arm thus formed can be seen in an autosome in metaphase preparations. This last circumstance has given us the opportunity to observe in salivary gland preparation the translocated heterochromatic part of the *Y*-chromosome. It can be seen at the end of the proximal part of IIR. If therefore, this heterochromatic part is represented by some sort of structure, this structure must be located at the end of the proximal part of IIR.

At first, all attempts to find any such structure were unsuccessful. However, trials of culture media were made to secure better development of *D. buscki* larvae, and this was responsible for final success. In one preparation, a unique type of chromosome element was found at the proximal end of IIR. The nature of this element could not be in doubt: it was in fact the heterochromatic part of the Y-chromosome, hitherto considered invisible. The most striking feature of this structure was its much greater transparency (as compared with the euchromatic portions), with pale bands, which are frequently dotted. In later work, it was often

possible to obtain preparations showing this element, but usually the structure hardly stains at all and could only be seen because of differences in refractive index. However, among a great many preparations, a few were obtained which showed the entire element and many of the features of its structure (see figure 1).

The length of the translocated *Y*-chromosome portion is about $^3/_5$ or $^3/_4$ of the length of the *X*-chromosome, or of any of the other elements of the salivary gland chromosomes. Its width is about equal to that of ordinary paired chromosomes. The distal end, or the tip, has no resemblance to any of the other salivary chromosomes of *D. buscki*. The bands are pale, difficult to see, often dotted, and all of them are single (dotting is not a constant character; in some preparations bands appear continuous). The spacing of the bands along the length of the chromosome is about the same as that of the bands of the euchromatic parts of other chromosomes. Because of the generally uniform staining of the individual bands, one gets an impression that they are also more uniformly distributed along the length. It should be noted that observation of the entire length of this chromosome is very difficult, because its visibility depends on the background on which it happens to lie. The best background is a uniform layer of cytoplasm; in places where there is no cytoplasm, the chromosome cannot be seen. By the least pressure on the cover slip, this chromosome is very easily deformed and disintegrated, leaving no visible trace, indicating an exceedingly fragile structure. Presumably for these reasons it is extremely difficult to observe the structure of the *Y*-chromosome in individuals without the translocation.

*Discussion.*—The original concept of heterochromatic chromosomes or chromosome parts as having no structure, concentrated as an amorphous mass in the so-called chromocenter, was radically modified by later investigators. In many chromosomes, genetically inert regions were demonstrated near the centromeres. Prokofyeva[6, 7] showed that such inert regions occurred not only near the centromeres but were also intercalated among euchromatic regions. The same author was first able to observe the *Y*-chromosome in salivary gland cells.[8] In *D. melanogaster* it can be seen as a short element containing some 8–10 very thin bands. This small *Y*-chromosome is usually localized at the periphery of the chromocenter, and often pairs with the inert region of the centromere end of the *X*-chromosome.[9] And yet, according to Prokofyeva, the observed segment of the *Y* is only its genetically active part, the remainder being invisible. Bauer[10] observed the *Y*-chromosome of *D. pseudoobscura* as a bundle of chromonemata, with chromioles (the so-called loose heterochromatin). He was unable to see a *Y*-chromosome in species of *Drosophila* other than *D. melanogaster* and *D. pseudoobscura*. In *D. miranda*, MacKnight[11] demonstrated the *Y*-chromosome as a complicated structure localized at

the center of the nucleus, together with the chromocenter. The peculiarity of the structure of the *Y* of this species is that it includes numerous sections of euchromatic material, originally parts of the third chromosome which has become involved in the sex-determining mechanism. These have in the course of time been much modified by fragmentation. Between the euchromatic segments are heterochromatic masses having no definite structure. In the *D. nebulosa*, according to Pavan,[12] the *Y*-chromosome is represented by a few chromomeres.

The inert part of the *Y*-chromosome of *D. buscki* which has the definite structure described above is unique. First of all, the translocation having $^2/_5$ or $^3/_4$ of the length of the *X*-chromosome makes us suppose that the entire *Y*-chromosome in salivaries is proportionally as long as it is at metaphase; that is, the *Y* is about equal in length to the *X*. This conclusion conflicts with the suppositions of Muller and Gershenson[13] and Prokofyeva[9] who suggested that the metaphase *Y*-chromosome consists of heterochromatic blocks invisible in cells of the salivary glands, or alternatively that it is unspiralized in metaphases. On the other hand the demonstration of a definite structure in the inert Y-chromosome confirms the conclusion of Muller and Prokofyeva[14] concerning the degree of resemblance between euchromatic and heterochromatic segments. We might say that the external appearance of the heterochromatic part of the *Y*-chromosome in *D. buscki* seems to be a replica of a normal euchromatic chromosome. But these authors made conclusions about the structure of heterochromatic chromosomes in general, on the basis of such small segments as had not yet fully lost genetic activity, and which perhaps for that reason had not yet lost all structural organization. Similarly we might suppose that the *Y*-chromosome of *D. buscki* is an example of such an element, at an intermediate stage in the process of inactivation.

Two facts are relevant to the above problem. First, the presence in *XXY* females of an entire *Y*-chromosome, including its active euchromatic part, in no way modifies their appearance or viability. Second, males lacking almost the entire long left arm of the *Y*-chromosome are fully viable. Hypoploid males of our mutant line *Curled-Y* thus behave, as far as the viability and the phenotype are concerned, exactly like *XO*, *XY'* and *XY*$^{9}$ of *D. melanogaster*. One may conclude then that the degree of genetic inactivation of the left arm of the *Y*-chromosome in *D. buscki* approaches that found in *D. melanogaster*. But the question still remains open why in *D. buscki* the *Y*-chromosome should still be visible in salivary gland cells even though difficult to observe, while in *D. melanogaster* only an insignificant element of it remains, and in other species are even completely absent.

*Summary.*—A translocation between the *Y*-chromosome and an autosome is described in *D. buscki*. The translocation provides a means of

observing in salivary gland preparations the structure of a considerable part of the heterochromatic region of the *Y*-chromosome. This inert segment resembles a euchromatic chromosome in size and in having clear-cut bands, but it is extremely transparent and takes up almost no stain. The bearing of this on the genetic nature of inert chromosomes is discussed.

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# EXPERIMENTS WITH THE CHEMOSTAT ON SPONTANEOUS MUTATIONS OF BACTERIA

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*Introduction.*—All bacteria require for growth the presence of certain inorganic chemical components in the nutrient, such as potassium, phosphorus, sulphur, etc., and with a few exceptions all bacteria require an energy-yielding carbon source, such as, for instance, glucose or lactate, etc. In addition to these elements or simple compounds, certain bacteria require more complex compounds, for instance an amino acid, which they are not capable of synthesizing. For the purposes of this presentation, any of the chemical compounds which a given strain of bacteria requires for its growth will be called a "growth factor."

In general, the growth rate of a bacterial strain may be within very wide limits independent of the concentration of a given growth factor; but since at zero concentration the growth rate is zero, there must of necessity exist, at sufficiently low concentrations of the growth factor, a region in which the growth rate falls with falling concentration of the growth factor. It therefore should be possible to maintain a bacterial population over an indefinite period of time growing at a rate considerably lower than normal simply by maintaining the concentration of one growth factor—the controlling growth factor—at a sufficiently low value, while the concentrations of all other growth factors may at the same time be maintained at high values.

We shall describe further below a device for maintaining in this manner, over a long period of time, a bacterial population in the growth phase at a reduced growth rate and shall refer to it as the Chemostat.

If the growth rate of a bacterial population is reduced, it is not *a priori* clear whether the growth rate of the individual cells which constitute the population is uniformly reduced or whether a fraction of the total cell population has ceased to grow and is in a sort of lag phase, while the rest keeps growing at an undiminished rate. We believe that under the conditions of our experiments, to be described below, we are dealing with the slowing of the growth rate of the individual cells rather than the cessation of growth of a fraction of the population.

By using an amino acid as the controlling growth factor we were able to force protein synthesis in the bacterial population to proceed at a rate ten times slower than at high concentrations of that amino acid. It appears that we are dealing here with a hitherto unexplored "state" of a bacterial population—a state of reduced growth rate under the control of a suitably chosen growth factor.

The study of this "slow-growth-phase" in the Chemostat promises to yield information of some value on metabolism, regulatory processes, adaptations and mutations of micro-organisms; the present paper, however, is concerned only with the study of spontaneous mutations in bacteria.

There is a well-known mutant of the B strain of coli, B/1, which is resistant to the bacterial virus $T_1$, sensitive to the bacterial virus $T_6$, and which requires tryptophane as a growth factor. We used this strain and mutants derived from it in all of our experiments here reported. As a nutrient medium we used a simple synthetic lactate medium (Friedlein medium) with tryptophane added. As the controlling growth factor, we used either lactate or tryptophane.

*Experiments on Growth Rates at Low Tryptophane Concentrations.*—In order to determine the growth rate of B/1 as a function of the tryptophane concentration (at high lactate concentrations) we made a series of experiments in which we incubated at 37° at different initial tryptophane concentrations $c$, flasks inoculated with about 100 bacteria per cc. and obtained growth curves by determining (by means of colony counts) the number of viable bacteria as a function of time. Because the bacteria take up tryptophane, the tryptophane concentration $c$ decreases during the growth of the culture and the growth rate for the initial tryptophane concentration $c$ must therefore be taken from the early part of the growth curve.

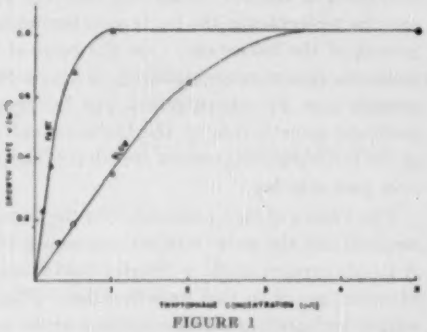

FIGURE 1

Experiment of September 18, 1950, at 37°C. The curve marked SLOW relates to strain B/1 and the curve marked FAST relates to B/1/f.

The growth rate $\alpha$ is defined by

$$\alpha = \frac{1}{n}\frac{dn}{dt}$$

where $n$ is the number of bacteria per cc. The reciprocal value, $\tau = \frac{1}{\alpha}$, we shall designate as the "generation time." From the generation time thus defined, we obtain the time between two successive cell divisions by multiplying by ln 2.

In figure 1 the curve marked "slow" shows the growth rate $\alpha$ as a function

of the tryptophane concentration $c$ for 37°. At low tryptophane concentrations $c$, the growth rate at first rises proportionately with the concentration; with increasing concentrations, however, the growth rate approaches a limit and for concentrations above 10 $\gamma$/l. (micrograms per liter) the growth rate is no longer appreciably different from its highest attainable value. This highest value corresponds to a generation time of $\tau = 70$ min. One half of the highest value is reached at a tryptophane concentration of about $c = 1$ $\gamma$/l. This concentration corresponds to about three molecules of tryptophane per $10^{-12}$ cc. (The volume of one bacterium is about $10^{-13}$ cc.)

The proportionality of the growth rate with the concentration of tryptophane at low concentrations becomes understandable if we assume that the uptake and utilization of tryptophane by the bacterium requires that a tryptophane molecule interact with a molecule of a certain enzyme contained in the bacterium and that the uptake of tryptophane by these enzyme molecules in the bacterium becomes the rate-limiting factor for the growth of the bacterium. On the basis of this argument, we believe that down to as low concentrations of tryptophane as the proportionality of growth rate to concentration can be experimentally demonstrated, the observed growth rate of the bacterial culture represents the growth rate of the individual bacterium and that no appreciable fraction of the population goes into lag.

*The Theory of the Chemostat.*—In the Chemostat, we have a vessel (which we shall call the growth tube) containing $V$ cc. of a suspension of bacteria. A steady stream of the nutrient liquid flows from a storage tank at the rate of $w$ cc./sec. into this growth tube. The content of the growth tube is stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the growth tube at all times. An overflow sets the level of the liquid in the growth tube, and through that overflow the bacterial suspension will leave the growth tube at the same rate at which fresh nutrient enters the growth tube from the storage tank.

After a certain time of such operation, at a fixed temperature, a stationary state is reached in the growth tube. We are interested in this stationary state in the particular case in which the growth rate of the bacteria is determined by the concentration in the growth tube of a single growth factor (in our specific case tryptophane). By this we mean that the concentration of a single growth factor (tryptophane) in the growth tube is so low that a small change in it appreciably affects the growth rate of the bacteria, and at the same time the concentration of all other growth factors in the growth tube is so high that a small change in them has no appreciable effect on the growth rate of the bacteria. As we shall show, under these conditions the concentration $c$ of the growth factor in the growth tube *in the stationary state*, for a fixed flow rate $w$, will be independent of the

concentration $a$ of this growth factor in the nutrient liquid in the storage tank.

In order to see this, we have to consider the following:

1. For zero flow rate of the nutrient ($w = 0$), the bacterial concentration $n$ would rise in the growth tube according to $\frac{1}{n}\frac{dn}{dt} = \alpha(c)$, where $\alpha$ is the growth rate which, according to our premise, is a function of the concentration, $c$, of the growth factor.

2. In the absence of *growth*, the bacterial concentration in the growth tube would decrease for a given flow rate $w$ according to the formula

$$\frac{1}{n}\frac{dn}{dt} = \frac{-w}{V}$$

where $\frac{w}{V} = \beta$ may be called the "washing-out rate" of the growth tube, and $\frac{1}{\beta}$ the washing-out time.

After a while, for any given flow rate $w$, a stationary state will be reached in the Chemostat at which the growth rate $\alpha$ will be equal to the washing-out rate $\beta$ $\left(\text{and the generation time } \tau \text{ equal to the washing-out time } \frac{1}{\beta}\right)$, i.e.,

$$\alpha(c) = \beta = \frac{w}{V}; \qquad \tau = \frac{1}{\beta} = \frac{V}{w}. \tag{1}$$

Thus, in the stationary state for any fixed flow rate $w$, the growth rate $\alpha$ is fixed; since $\alpha$ is a function of the concentration $c$ in the growth tube, it follows that $c$ is also fixed and independent of the concentration $a$ of the growth factor in the storage tank.

It may be asked what is the mechanism by which, for different values of $a$ but the same flow rate $w$, the same concentration $c$ establishes itself in the growth tube in the stationary state. Clearly what happens is this: Suppose that, for a certain concentration $a_1$ of the growth factor in the storage tank, a stationary state with the concentration $c$ in the growth tube has established itself and subsequently the concentration of the growth factor in the storage tank is suddenly raised to a higher value $a_2$. When this change is made, the concentration $c$ in the growth tube will at first rise and along with it will rise $\alpha$, the growth rate of the bacteria, which is a function of $c$. The concentration of the bacteria in the growth tube will thus start to increase, and therefore the bacteria will take up the growth factor in the growth tube at an increased rate. As the increase of the bacterial concentration continues, the growth rate of the bacteria will, after a while, begin to fall and will continue to fall until a new stationary state is reached

at which the bacteria again grow at the same rate at which they are washed out, i.e., for which again we have $\alpha = \frac{w}{V}$. When this state is reached, the concentration of the growth factor in the growth tube is again down to the same value $c$ which it had before the concentration of the growth factor in the storage tank was raised from $a_1$ to $a_2$, while the bacterial density is now higher.

In the stationary state the tryptophane balance requires that the following equation hold:

$$a = c + n \frac{V}{w} F(c) \tag{2}$$

or

$$a = c + n \frac{F(c)}{\alpha(c)} \tag{3}$$

where $F(c)$ gives in grams per second the amount of the growth factor which one bacterium takes up per second.

As can be easily seen, the amount $Q$ of the growth factor that is taken up per bacterium produced is given by

$$Q = \frac{F(c)}{\alpha(c)}$$

so that, for the stationary state, we may also write

$$a = c + nQ \quad \text{or} \quad n = \frac{a - c}{Q} \tag{4}$$

and for the $c \ll a$ we may write

$$n = \frac{a}{Q}. \tag{5}$$

*The Use of Tryptophane as the Controlling Growth Factor.*—Since in the stationary state the tryptophane concentration in the growth tube of the Chemostat is always below 10 $\gamma$/l. whenever the generation time is appreciably above 70 min., we may use the approximation given in equation (5) whenever the tryptophane concentration $a$ in the storage tank is above 100 $\gamma$/l.

In order to determine the amount of tryptophane, $Q$, taken up per bacterium produced, we grew bacterial cultures in lactate medium with varied amounts of tryptophane added. We found that if the initial tryptophane concentration is kept below 10 $\gamma$/l., then the amount of tryptophane taken up per bacterium produced is not dependent on the tryptophane concentration and has a value of $Q = 2 \times 10^{-15}$ gm. At higher tryptophane concentrations, however, more tryptophane is used up per bacterium produced.

From equation (5), using the value of $Q = 2 \times 10^{-14}$ gm. we obtain $n = 5 \times 10^7$/cc. for $a = 100$ $\gamma$/l. and we obtain $n = 5 \times 10^8$/cc. for $a = 1000$ $\gamma$/l.

From this, it may be seen that, by choosing suitable values for $a$ and $w$, we may vary over a wide range, independently of each other, the bacterial concentration $n$ and the tryptophane concentration $c$.

When we grew B/1 in a Chemostat ($V = 20$ cc.) for ten days at 37° at a generation time of $\tau = 2$ hrs. and at a bacterial density of $5 \times 10^8$/cc., we found that a change from the original bacterial strain, B/1, had taken place. The new strain, which we shall designate as B/1/f, differs from the original strain only inasmuch as it grows, at very low tryptophane concentrations, about five times as fast as the original strain. The growth rate at higher tryptophane concentrations is not perceptibly different, nor could we detect any other difference between the two strains. The curve marked "fast" in figure 1 gives the growth rate of the B/1/f strain as a function of the tryptophane concentration at 37°.

The ability of the B/1/f strain to grow faster at very low tryptophane concentrations gives it an advantage over the B/1 strain under the conditions prevailing in the growth tube of the Chemostat; and a mutant of this sort must, in time, displace the original strain of B/1.

Because in our experiments we would want to avoid—as much as possible—population changes of this type in the Chemostat, we used in all of our experiments reported below this new strain, B/1/f.

*Spontaneous Mutations in the Chemostat.*—If we keep a strain of bacteria growing in the Chemostat and through spontaneous mutations another bacterial strain is generated from it, then the bacterial density $n^*$ of the mutant strain should (for $n^* \ll n$) increase linearly with time, provided that, under the conditions prevailing in the Chemostat, the new strain has the same growth rate as the original strain, so that there is no selection either for or against the mutant. In the absence of selection we have

$$\frac{dn^*}{dt} = \frac{\lambda}{\tau} n \tag{6}$$

where $n^*$ is the density of the mutant population, $n$ is the density of the population of the parent strain and $\lambda$ the number of mutations produced per generation per bacterium. Equation (6) holds under the assumption that back mutations can be neglected. From (6), we obtain for $n^* \ll n$

$$\frac{n^*}{n} = \frac{\lambda}{\tau} t + \text{Const.} \tag{7}$$

From this it may be seen that—as stated above—the relative abundance of the mutants must increase linearly with time if there is no selection for or against the mutant.

If the growth rate of the mutant strain is smaller than the growth rate of the parent strain ($\alpha^* < \alpha$) so that there is selection against the mutant in the growth tube of the Chemostat, then the density $n^*$ of the mutant population should—after an initial rise—remain constant at the level given by

$$\frac{n^*}{n} = \frac{\alpha}{\alpha - \alpha^*} \lambda. \tag{8}$$

*Experiments on Spontaneous Mutations in the Chemostat.*—Of the various mutations occurring in a growing bacterial population, mutants resistant

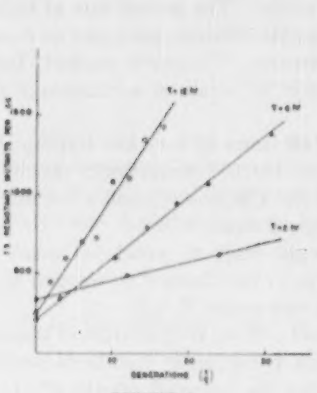

FIGURE 2

Experiments of May 3, 8, and 28, 1950, at 37°C. giving for strain B/1/f for three different values of the generation time the concentration of the mutants resistant to $T_6$, for a population density of $5 \times 10^8$ bacteria per cc.

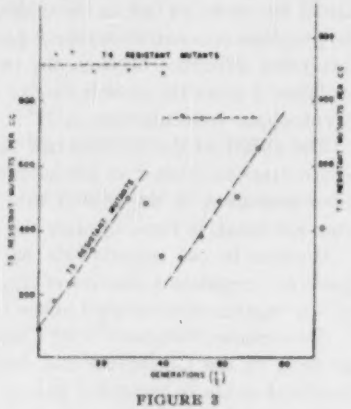

FIGURE 3

Experiment of July 19, 1950, at 37°C. giving for strain B/1/f the concentration of mutants resistant to $T_5$ (left-hand scale) and mutants resistant to $T_4$ (right-hand scale) for a population density of $2.5 \times 10^8$ bacteria per cc. In this experiment oxygen containing 0.25% $CO_2$ was used for aeration.

to a bacterial virus are perhaps the most easily scored with considerable accuracy. In our experiments we mostly worked with mutants of our coli strain which were resistant to the bacterial viruses $T_5$ or $T_4$.

When we grow the strain B/1/f in the Chemostat with a high concentration of tryptophane but a low concentration of lactate in the nutrient in the storage tank, so that lactate rather than tryptophane is the controlling growth factor, we find—after a short initial period—that the bacterial densities of the mutants resistant to $T_5$ or $T_6$ each remain at a constant level. These levels appear to correspond to a selection factor

$\frac{a - a^*}{a}$ of a few per cent.

We are inclined tentatively to assume that the behavior of these two mutants exemplifies the general rule that the vast majority of all the different mutational steps leading away from the wild type yield mutants which—under conditions of starvation for the carbon source—grow slower than the parent type.

On the other hand, if we grow our tryptophane-requiring strain in the Chemostat with a high concentration of lactate but a low concentration of tryptophane in the nutrient in the storage tank (so that tryptophane rather than lactate is the controlling growth factor) and if we run the Chemostat at a generation time well above 70 min. (the generation time at high tryptophane concentrations)—then there is no reason to expect mutants *in general* to grow appreciably slower than the parent strain, particularly if the growth of the parent strain is kept very slow by keeping the tryptophane concentration in the growth tube very low. In this case one would rather expect a mutation to affect the growth rate only if it affects the uptake or utilization of tryptophane by the bacterium or if the mutant is a very slow grower. Accordingly, we should, in general, expect the mutant population to increase linearly with time in the Chemostat when tryptophane is used as the controlling growth factor.

Figure 2 gives for 37° the experimental values for the bacterial density for the mutant population resistant to $T_5$ in the growth tube of the Chemostat as a function of the number of generations through which the parent strain has passed in the Chemostat. $\left(\text{Number of generations } g = \frac{t}{\tau}.\right)$ The three curves in the figure correspond to generation times of 2 hours, 6 hours and 12 hours. The slope of the straight lines gives $\lambda$, the mutation rate per generation, as $2.5 \times 10^{-8}$; $7.5 \times 10^{-8}$; and $15 \times 10^{-8}$ per bacterium. We see that the mutation rate per generation for $\tau = 6$ hours is three times as high and for $\tau = 12$ hours is six times as high as it is for $\tau = 2$ hours. Thus the mutation rate per generation is, in our experiment, not constant but increases proportionately with $\tau$ and what remains constant is the number of mutations produced per unit time per bacterium. According to the above figures, we have $\frac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium.

This result is not one that could have been foreseen. If mutants arose, for instance, as the result of some error in the process of gene duplication, then one would hardly expect the probability of a mutation occurring per cell division to be inversely proportionate to the rate of growth.

If the processes of mutation could be considered as a monomolecular reaction—as had been once suggested by Delbruck and Timofeeff-Ressovsky—then, of course, the rate of mutation per unit time should be

constant. The rate $k$ of a monomolecular reaction is given by

$$k = Ae^{-W/RT}. \qquad (9)$$

The value of the constant $A$ can be calculated from the observed reaction rate $k$ and the heat of activation $W$ (which can be obtained by determining the temperature coefficient of the reaction).

Using the Chemostat, we have determined the rate of mutation to resistance to $T_5$ at 25° (for $\tau$ = 6 hrs. and $\tau$ = 12 hrs.) and found it to be about one half of the mutation rate at 37°. From this value and the mutation rate of $\frac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium at 37° we compute $A \approx 10^{13}$ per sec.

In a condensed system, such as an aqueous solution, $A$ has been found to lie between $10^{8}$ and $10^{14}$ per sec. for known monomolecular reactions. Therefore if the mutation studied by us were due to a monomolecular reaction, it would have an $A$ value $10^{3}$ times lower than the lowest value so far found.

The density of the mutants resistant to the bacterial virus $T_6$ in the Chemostat, with tryptophane as the controlling growth factor, also appears to rise linearly with time for $\tau$ = 2 hours, $\tau$ = 6 hours and $\tau$ = 12 hours, but our results so far are not sufficiently accurate to say whether this mutation also occurs at a constant rate per unit time for different generation times $\tau$. The temperature coefficient of the mutation rate appears to be very low, but again this conclusion must await more accurate experiments.

The result obtained for mutation to resistance to the virus $T_5$, showing that this mutation occurs at a constant rate per unit time up to a generation time of $\tau$ = 12 hours, raises the question whether this is generally true of spontaneous bacterial mutations or whether we are dealing in our case with certain exceptional circumstances. Clearly, a number of different mutations will have to be examined, different amino acids will have to be used as the controlling growth factor and other conditions will have to be varied before one would draw the far-reaching conclusion that our observation on mutation to resistance to the virus $T_5$ exemplifies a general rule.

*Mutants Resistant to $T_4$.*—We find that mutants resistant to $T_4$ are selected against in the Chemostat when grown either with lactate or with tryptophane as the controlling growth factor, i.e., the number of mutants remains—after an initial rise—at a fixed level.

It is known that of the different mutants of the B strain of coli which are resistant to the virus $T_4$, the most frequent one is also resistant to the viruses $T_3$ and $T_7$ and that this mutant is a very slow grower under ordinary conditions of culture. It is conceivable that this might explain why the mutants resistant to $T_4$ are selected against in the Chemostat even when

the bacterial population grows under tryptophane control and at a much reduced rate.

*Manifestation of "Evolution" in the Chemostat.*—If a bacterial strain is grown over a long period of time in the Chemostat, from time to time a mutant might arise which grows faster, under the conditions prevailing in the Chemostat, than the parent strain. If this happens, practically the entire bacterial population in the Chemostat will change over from the parent strain to the new strain. We have discussed one change-over of this sort, i.e., the change-over from the strain B/1 to the strain B/1/f. There is no reason to believe, however, that no further change-over may take place when we start out with B/1/f as the parent strain and continue to grow it in the Chemostat over a long period of time.

We have seen that the mutants resistant to $T_5$ accumulate in the Chemostat and that their number rises linearly with the number of generations, giving a straight line, the slope of which is given by $\lambda$. If now at a certain time the population changes over in the Chemostat from the parent strain to a faster-growing strain, the accumulated mutants resistant to the bacterial virus $T_5$ which were derived from the parent strain should disappear from the Chemostat along with the parent strain. This should lead to a fall in the number of mutants resistant to the bacterial virus $T_5$ during a change-over from the parent strain to the faster-growing strain. After the change-over to the new strain, the concentration of the mutants resistant to $T_5$ may be expected again to increase linearly with the number of generations, giving a straight line which has the same slope as before the change-over, because the new strain which displaces the parent strain may be expected to mutate to resistance to $T_5$ at an unchanged rate $\lambda$.

Thus, we may in general expect, when a change-over in the population takes place, the concentration of the mutants resistant to $T_5$ to shift from one straight line which lies higher to another, which lies lower. The magnitude of this shift may be somewhat different from experiment to experiment, depending on when mutants resistant to $T_5$ happen to make their first appearance in the population of the new strain.

At the outset, the bacteria belonging to the new strain will be few in number but their number will increase exponentially with the number of generations until—at the time of the change-over—the bacteria belonging to the new strain become an appreciable fraction of the total population. If the mutation rate to resistance to $T_5$ is of the order of magnitude of $10^{-8}$, then it is unlikely that such a mutant should appear in the population of the new strain until its population has reached perhaps $10^7$. However, because an element of chance is involved, occasionally a mutant resistant to $T_5$ may appear earlier and, if that happens, the "shift" associated with the change-over will be smaller and in principle it might even be negative.

If a bacterial population remains growing in the Chemostat for a sufficiently long time, a number of such change-overs might take place. Each

such step in the evolution of the bacterial strain in the Chemostat may be expected to manifest itself in a shift in the ascending straight line curve of the $T_5$ resistant mutants.

As we have seen, the mutants resistant to $T_4$ remain—apart from an initial rise—at a constant level in the Chemostat. However, when the bacterial population in the Chemostat changes over from a parent strain to a new strain, the $T_4$ resistant mutants might change over from one level to another, because the selection against the two strains might be different.

Figure 3 shows, for mutants resistant to $T_5$ and for mutants resistant to $T_4$, the number of mutants as a function of the number of generations $\frac{t}{\tau}$ in a Chemostat which was run for 300 hours at $\tau = 4$ hours with tryptophane as the controlling growth factor.

It may be seen that these two curves show a population change-over of the type just described. The curve for the $T_5$ resistant mutants shows a shift, $P$, of $P = 32$ generations.

A number of shifts of this type were observed in different experiments. We verified that these "shifts" represent population change-overs by showing in one case that (under the conditions prevailing in the chemostat) bacteria taken from the Chemostat before the change-over in fact grow slower than bacteria taken from the Chemostat after the change-over.

In order to show this, we took from the Chemostat before the change-over a bacterium resistant to $T_5$ and after the change-over a bacterium sensitive to $T_5$ and inoculated a *second* Chemostat (operated under identical conditions) with a 50-50 mixture of these two strains. We then found that the relative abundance of the resistant strain rapidly diminished. In the corresponding control experiment we took a sensitive bacterium from the Chemostat before the population change-over and a resistant one after the population change-over and again found that the strain prevalent before the change-over (this time the sensitive one) was the slower grower.

In the later stages of the change-over the concentration $x$ of the original strain falls off exponentially with the number of generations, $g = \frac{t}{\tau}$, so that we may write $x = Ce^{-g/\gamma}$. In our experiment we obtained for $\gamma$ a value of $\gamma = 3.25$.

It should be noted that the value of $\gamma$ can be read also directly (though not accurately) from the curve, which gives the concentration $n^*$ of the resistant mutants as the function of $g$, the number of generations. During the change-over the concentration $c$ of the tryptophane in the growth tube goes over from an initial value $c_1$ to a final, lower value $c_2$ and it can be shown that for the midpoint of the change-over at which $c = \frac{c_1 + c_2}{2}$ we have

$$\gamma = \frac{P/4}{1 - \frac{1}{\lambda n}\frac{dn^*}{dg}} + \frac{1}{2} \qquad (10)$$

where $P$ is the magnitude of the shift expressed in the number of generations by which the ascending straight line of the resistant mutants is shifted in the change-over. This formula holds only if $\tau$ is large so that the rate of growth of the bacteria in the Chemostat is proportionate to the tryptophane concentration $c$. Because the exact position on the curve of the midpoint of the change-over on the curve $n^*$ is not known, this formula can give only a rough indication for the value of $\gamma$.

In our case, the estimate based on it gave for $\gamma$ a value of $\gamma = 2.4$ in place of the directly observed value of $\gamma = 3.25$. Within the limits of the accuracy of our curve for $n^*$ these two values are consistent with each other.

Population change-overs manifesting themselves in a shift in the ascending straight line of the $T_4$ resistant mutants occurred in every experiment carried at $\tau = 4$ hrs. beyond the 50th generation. In an experiment carried to the 450th generation at a bacterial density of $2.5 \times 10^8$/cc., a number of such shifts occurred, the last one at about the 350th generation. (In the course of this experiment the mutants resistant to $T_4$ rose twice from a low level to a high peak, the first of which reached $4.6 \times 10^4$ and the second $4.5 \times 10^6$ mutants per cc. This phenomenon is now being investigated.)

It may be said that our strain, if grown in the Chemostat at low tryptophane concentration for a long period of time, undergoes a number of mutational steps, each one leading to a strain more "fit" than the previous one, and that each step in this process of evolution becomes manifest through the shifts appearing in the curve of the mutants resistant to $T_4$.

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## *THE ANALYSIS OF A CASE OF CROSS-STERILITY IN MAIZE*

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The male gametophyte of maize is extremely sensitive to chromosomal unbalance. Duplications and deficiencies are almost always accompanied by reduced pollen transmission. The functioning of the male gametophyte is also known to be affected by a number of genic factors. The most thoroughly studied locus and probably the most interesting is *Ga*$_1$ on chromosome 4. First detected by Correns[9] because of aberrant $F_2$ ratios for sugary-starchy, this factor was subsequently studied by Jones,[8, 10]

Emerson,[6, 7] Mangelsdorf,[10] and Demerec.[4] Emerson established that the differential fertilization was not due to the sugary gene itself but to some other gene with which it was linked.

$Ga_1$ is comparable to the self-sterility genes found in strictly cross-fertilized plants such as Nicotiana, Oenothera, Trifolium, etc., in that there is interaction between the stylar tissue and the male gametophyte. When the female parent is homozygous recessive $ga_1$, $ga_1$ pollen can compete successfully against $Ga_1$ and fertilize half of the ovules. However, if the plant used as the egg parent is heterozygous or homozygous $Ga_1$, the $ga_1$ pollen is a poor competitor and achieves fertilization in only 0–4% of the ovules. In the absence of competition, that is, if only $ga_1$ pollen is used in the cross, full seed set is obtained regardless of the genotype of the female parent.[7] Thus the incompatibility between $ga_1$ pollen and $Ga_1$ silk is not detectable in the absence of competing $Ga_1$ pollen. The purpose of this paper is to present evidence for the existence of a third allele at this locus.

TABLE 1

SUMMARY OF DATA SHOWING THE COMPETITIVE FUNCTIONING OF $Ga_1^s$, $Ga_1$, AND $ga_1$ ON STYLES OF VARIOUS GENOTYPES

| ITEM | GENOTYPIC CONSTITUTIONS | NUMBER OF KERNELS | | | % SUGARY |
|---|---|---|---|---|---|
| | | STARCHY | SUGARY | TOTAL | |
| 1 | $Ga_1^sSu/Ga_1su \times Ga_1^sSu/Ga_1su$ ♂ | 19,168 | 5865 | 25,033 | 23.4 |
| 2 | $Ga_1^sSu/ga_1su \times Ga_1^sSu/Ga_1su$ ♂ | 4,194 | 1300 | 5,494 | 23.6 |
| 3 | $Ga_1su/Ga_1su \times Ga_1^sSu/Ga_1su$ ♂ | 781 | 666 | 1,447 | 46.0 |
| 4 | $Ga_1su/ga_1Su \times Ga_1^sSu/Ga_1su$ ♂ | 4,624 | 1432 | 6,056 | 23.6 |
| 5 | $Ga_1^sSu/Ga_1su \times Ga_1^sSu/ga_1su$ ♂ | 1,548 | 221 | 1,769 | 12.5 |
| 6 | $Ga_1^sSu/ga_1su \times Ga_1^sSu/ga_1su$ ♂ | 4,142 | 611 | 4,753 | 12.9 |
| 7 | $Ga_1su/Ga_1su \times Ga_1^sSu/ga_1su$ ♂ | 237 | 86 | 323 | 26.6 |
| 8 | $Ga_1su/ga_1Su \times Ga_1^sSu/ga_1su$ ♂ | 3,866 | 675 | 4,541 | 14.9 |
| 9 | $ga_1su/ga_1su \times Ga_1^sSu/ga_1su$ ♂ | 412 | 408 | 820 | 49.8 |
| 10 | $Ga_1^sSu/ga_1su \times Ga_1su/ga_1Su$ ♂ | 2,096 | 1104 | 3,200 | 34.6 |

This allele, designated $Ga_1^s$ since its effect is stronger than $Ga_1$, was found by Dr. M. M. Rhoades who turned it over to the author for further analysis.

The most important difference between $Ga_1$ and $Ga_1^s$ is that $ga_1$ pollen completely fails to function on styles homozygous for $Ga_1^s$ even in the absence of competing pollen. From 14 such crosses only one seed was obtained; this could have been the result of contamination. Crosses of $ga_1$ pollen on styles heterozygous for $Ga_1^s$ yield a partial seed set. Less seed is set when the female parent is heterozygous $Ga_1^sGa_1$ than when a heterozygous $Ga_1^sga_1$ plant is used. The total amount of seed produced in such crosses varies greatly from plant to plant.

The linkage relation of $Ga_1^s$ with *Su* and its interaction with $Ga_1$ suggest that it is an allele of $Ga_1$. Both $Ga_1$ and $Ga_1^s$ exhibit selective fertilization in competing with $ga_1$ only when the female parent in the cross carries one of the dominant alleles. No selective fertilization occurs when the female

parent is homozygous $ga_1$ (table 1, item 9). On the assumption that $Ga_1$ and $Ga_1^s$ represent two independent loci, and that $ga_1$ and $ga^s$ gametes can compete successfully on respective recessive styles against pollen carrying the dominant allele, no differential fertilization should result from crosses of $ga_1ga_1Ga^sGa^s$ by $Ga_1ga_1ga^sga^s$ ♂ and of $Ga_1Ga_1ga^sga^s$ by $ga_1ga_1Ga^sga^s$ ♂. Moreover, pollen from $Ga_1Ga_1ga^sga^s$ individuals should give no seed set when crossed on $ga_1ga_1Ga^sGa^s$ plants. This, however, was not found to be true; in the crosses of $Ga_1^sga_1 \times Ga_1ga_1$ ♂ and $Ga_1^sGa_1 \times Ga_1^sga_1$ ♂, $ga_1$ pollen is a very poor competitor (table 1). The cross $Ga_1^sGa_1^s \times Ga_1Ga_1$ ♂ yielded a full seed set. These data negate the hypothesis that $Ga_1^s$ and $Ga_1$ represent two independent loci but do not rule out the possibility of an interaction between the two. One might propose that the presence of $Ga_1$ in the $Ga_1ga^s$ gamete permits the functioning on $Ga^s$ styles of an otherwise handicapped pollen grain. It seems unlikely, however, that such interaction of loci would exist in view of the specific action of the self-sterility alleles in Nicotiana. East and Yarnell[8] have shown that there are at least 15 alleles at the self-sterility locus. Each allele is so specific in its action that the rate of growth of a pollen tube containing any one of them is determined solely by the presence of that particular allele in the diploid sporophytic tissue of the style and is completely independent of any other which may also be present. This specificity breaks down to some extent in polyploids of Oenothera[9] and white clover.[1]

Linkage studies have shown that the recombination values obtained for $Ga_1^s$ and *Su* are very close to those found by Emerson for $Ga_1$ and *Su*. A recombination value of 30% between the latter was calculated on the basis of 0% functioning of $ga_1$ pollen. This value was obtained by determining the percentage of sugary kernels resulting from a cross such as $Ga_1su/Ga_1su \times Ga_1Su/ga_1su$ ♂. If no $ga_1$ pollen functions, all of the sugary kernels result from crossing over between $Ga_1$ and *Su*, and the percentage of sugary kernels on the ears represents the crossover frequency. However, if some $ga_1$ pollen does function, then not all of the sugary kernels are crossovers and the recombination value is somewhat lower. Emerson showed that $ga_1$ pollen is responsible for the fertilization of from 0 to 9% of the ovules with the mean at 4%. On the basis of 4% functioning of $ga_1$ pollen he calculated a recombination value of 27.8%. When the $Ga_1^s$ allele was substituted for $Ga_1$ in similar crosses, a percentage of 25.8% sugary kernels was obtained (table 1, item 6). This value, which represents the recombination between $Ga_1^s$ and *Su*, agrees well with that calculated by Emerson for $Ga_1$-*Su*.

That the discrepancy between the recombination values of 30% for $Ga_1$-*Su* and 25.8% for $Ga_1^s$-*Su* is due to the amount of $ga_1$ pollen which functions is best shown by the following experiment. $Ga_1^sSu/ga_1su$ plants were used as the pollen parents in crosses with (1) $Ga_1su/ga_1Su$, and (2)

$Ga_1^sSu/ga_1su$ plants. Since the same male parent was used in both crosses the amount of crossing over between $Ga_1^s$ and *Su* would be identical and any significant difference in the percentage of sugary kernels resulting from these crosses would be due to the functioning of $ga_1$ pollen. The data are given as items 6 and 8 in table 1. The crosses involving $Ga_1$ gave 14.9% sugary kernels while 12.9% sugary seeds were obtained when $Ga_1^s$ was involved. The difference between 12.9 and 14.9% is significant at the 1% level and may logically be ascribed to the differential functioning of $ga_1$ pollen on $Ga_1$ and $Ga_1^s$ silks. The value of 14.9% is very close to the 15.1% found by Emerson in similar crosses using $Ga_1$. It follows that $ga_1$ pollen occasionally functions on $Ga_1$ styles even when competing against $Ga_1^s$ pollen.

The close agreement in the amount of crossing over between $Ga_1^s$ and $Ga_1$ with *Su* is indicative of an allelic relationship between the two genes. However, a possible alternative must be considered—namely, that $Ga_1^s$ and $Ga_1$ represent two loci lying equidistant from (in terms of crossover units), but on opposite sides of, Su. Such a condition would place the two genes more than 50 map units apart.

TABLE 2

DISTRIBUTION OF EARS RESULTING FROM THE SELF-POLLINATION OF PLANTS DERIVED FROM THE CROSS $Ga_1^sSu/Ga_1su \times ga_1su$ ♂

| | SUGARY KERNELS, % | | | |
|---|---|---|---|---|
| FAMILY | 8–12 | 13–17 | 18–22 | 23–27 |
| 1360 | 0 | 4 | 3 | 0 |
| 1361 | 6 | 24 | 7 | 0 |
| 1362 | 1 | 7 | 3 | 0 |

In order to test this hypothesis the following crosses were made: $(Ga_1^sGa_1^sSuSu \times Ga_1Ga_1susu$ ♂$) \times ga_1ga_1susu$ ♂. The sugary seeds were discarded since a heterozygous *Su/su* condition is necessary to detect the presence or absence of the gamete factor. On the assumption that two loci are involved, the $F_1$ would be genotypically $ga_1SuGa^s/Ga_1suga^s$. The second cross would yield four classes of starchy kernels: (1) $ga_1SuGa^s/ga_1suga^s$, (2) $Ga_1SuGa^s/ga^ssuga_1$, (3) $ga_1Suga^s/ga^ssuga_1$, and (4) $Ga_1Suga^s/ga^ssuga_1$. The first class is a non-crossover; the second, a single crossover in region (1) ($Ga_1$-*Su*); the third, a single crossover in region (2) (*Su*-$Ga^s$); and the fourth represents a double crossover in regions (1) and (2). Classes 1, 2 and 4 are difficult to distinguish and when self-pollinated would yield only 12–15% sugary seeds due to the linkage of *Su* with the dominant gamete factor. However, class 3, comprising almost 25% of all the starchy seeds would be detected since in the absence of either $Ga_1$ or $Ga^s$ normal Mendelian ratios of starchy-sugary kernels, i.e., 25% sugary, should result upon self-pollination. On the other hand, if $Ga^s$ and $Ga_1$, are alleles of the same locus, the cross of the $F_1$ with $ga_1su$ should give only 2 classes of

starchy seeds, $Ga_1^sSu/ga_1su$ and $Ga_1Su/ga_1su$. On this basis, no 3:1 starchy-sugary ratios would be expected on self-pollinated ears. The results are summarized in table 2. Since only low sugary ratios resulted, it is apparent that $Ga_1^s$ and $Ga_1$ are not separate loci located on opposite sides of *Su*.

When $Ga_1$ and $Ga_1^s$ pollen compete, the latter has a definite advantage in achieving fertilization on both $Ga_1$ and $Ga_1^s$ styles. Tests to determine the degree of selective fertilization were made using female parents of four genetic constitutions: (1) $Ga_1^sSu/Ga_1su$, (2) $Ga_1^sSu/ga_1su$, (3) $Ga_1su/Ga_1su$ and (4) $Ga_1su/ga_1Su$. The pollen parents were $Ga_1su/Ga_1^sSu$. The results are given in table 1. In the absence of any differential fertilization the crosses with the heterozygotes should produce 25% sugary kernels and the backcrosses to homozygous sugary plants should yield equal numbers of both types of seeds. However the 23.5% sugary kernels in the $F_2$ population and the 46% sugary in the backcrosses were significantly different at the 1% level from the expected percentages. Assuming 26% recombination between $Ga_1$ and *Su* it may be calculated that $Ga_1^s$ pollen fertilized 56% of the ovules in competition with $Ga_1$ pollen.

The manner in which selective fertilization is accomplished has not been established. Jones,[6] working with $Ga_1$, compared the ratios of sugary to starchy kernels on the butt and tip ends of the ears and concluded that the causal agent was differential pollen-tube growth. Emerson[7] was not able to confirm this. Demerec[4] claimed that selective fertilization was due to cross sterility rather than to competition between the pollen tubes. However, from his data it would appear that Demerec was working with a different allele from that studied by Jones and Emerson. Demerec's gamete factor is analogous to the $Ga_1^s$ allele reported in this paper in that $ga_1$ pollen does not function well in styles which carry the dominant allele even in the absence of competing pollen. The 12.4% sugary kernels which he obtained in the $F_2$ of sweet corn by plants showing the cross-sterility compares well with the reported 12.9% sugary resulting from the self-pollination of $Ga_1^sSu/ga_1su$ plants.

Demerec listed three possible explanations of this cross sterility.

(1) Inability of pollen with recessive gametophyte factor to germinate on silks having dominant allele.

(2) Inability of recessive pollen tubes to grow in silks with dominant allele.

(3) Inability of recessive pollen tubes to reach the ovules in the plant having the dominant allele.

In the case of $Ga_1^s$, it was possible to eliminate the first two causes experimentally. Plants of the constitution $Ga_1^sGa_1^s$ were pollinated with $ga_1$ pollen. Two hours after pollination the silks were removed, fixed in modified Carnoy solution and stained with propionic carmine. It was evident

that the pollen grains had germinated and that the pollen tubes had penetrated the stylar tissue.

Demerec's third suggestion remains as a possibility and a fourth may be added which is likewise untested. The failure of $ga_1$ pollen to fertilize $Ga_1^s$ plants may be independent of stylar interaction altogether, and may involve some abnormal reaction within the ovule, such as inability of the pollen tube to penetrate the embryo sac. None of these explanations, however, are applicable in the case of $Ga_1$-$ga_1$ competition since, in the absence of $Ga_1$ pollen, $ga_1$ gametes function normally on $Ga_1$ styles.

A number of cases of cross-sterility in maize may be due to the action of $Ga_1^s$. This is especially true of the cross-sterility found among varieties of popcorn.[2] Although the point of origin of the $Ga_1^s$ allele is not known, the fact that both $Ga_1$ and Demerec's cross sterility factor were found in popcorn supports this contention.

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## *THE USE OF SODIUM NUCLEATE IN THE STUDY OF THE MUTAGENIC ACTIVITY OF ACRIFLAVINE IN ESCHERICHIA COLI**

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In a previous study,[1] neutral acriflavine was shown to induce mutations from phage-sensitivity to phage-resistance in *Escherichia coli*. The present report concerns an improved method of investigating the mutagenic potency of certain compounds, using acriflavine as a model.

One of the difficulties encountered in working with acriflavine, as well as with numerous other compounds, was the tenacity with which the chemical remains bound to the treated cell. Ordinary washing methods were effective in removing acriflavine only to a limited degree, and thoroughly washed suspensions of treated bacteria were found to contain enough active chemical to limit subsequent growth, and to interfere with

the process of infection by bacteriophage. Another problem was the tendency of acriflavine to cause clumping of bacteria in dense cell suspensions. The agglutination could be controlled in part by adjustment of experimental conditions, but was extremely difficult to eliminate entirely. Since these complications, which were encountered with several other compounds, could operate to simulate mutagenicity, it seemed important to develop a method whereby they could be circumvented or overcome.

It has been reported that the antibacterial activity of acridines can be inhibited by the addition of nucleic acids and some of their derivatives,[2, 3] and the kinetics of this interaction have been described.[4] In these experiments, sodium ribonucleate was used to bring the exposure of bacteria to acriflavine to a sharp end point, to eliminate residual activity of this substance in the subsequent handling of the treated bacteria, and to prevent agglutination. In principle, this method is applicable to other antibacterial compounds for which more or less specific reversing agents are available.

*Material and Methods.*—Strain B/r of *E. coli* was used. The stock was carried on nutrient agar slants, and subcultured every two months. Inocula for experimental cultures were taken from a 24-hour culture grown in nutrient broth from about 100 cells.

Bacteriophage T1 was used in the isolation of mutants, and was kept in broth suspensions having a titer of about $10^{10}$ particles per ml.

Difco nutrient broth and Difco nutrient agar, each with 0.5% NaCl added, were used as culture media.

Neutral acriflavine was obtained from the National Aniline Division of the Allied Chemical and Dye Corporation, New York City. Solutions were made in distilled water, sterilized by boiling and used for no longer than three days. Sodium ribonucleate (yeast), obtained from Schwartz Laboratories, New York City, was dissolved in distilled water and sterilized by immersion in a boiling water-bath for fifteen minutes.

All assays were made by quantitative dilution and surface plating. Determinations of the frequency of phage-resistant mutants were made by spreading 0.1-ml. aliquots of culture on the surface of plates previously coated with about $10^9$ phage particles.

Incubation was at 37°C.

Each experimental series consisted of 20–50 tubes containing 5 ml. of broth to which acriflavine was added to give a concentration of 0.01%. Each tube was inoculated with about $10^7$ bacteria and incubated for 3–4 hours, or until survival would be expected to reach between $10^{-2}$ and $10^{-3}$. At this time, sodium nucleate was added to each tube, to give a concentration of 0.5%, and several tubes were assayed immediately to determine precisely the number of viable bacteria per culture. The cultures were then incubated for 18–24 hours, after which several were assayed

to determine the average titer, and an aliquot from each culture was plated with phage to obtain the number of T1-resistant mutants.

Controls consisted of 20–50 tubes containing 5 ml. of 0.01 acriflavine in broth, to each of which were added *simultaneously* sodium nucleate (final concentration 0.5%) and an inoculum of $10^4$–$10^5$ untreated bacteria. These cultures were incubated 18–24 hours, and assayed for titer and phage-resistant mutants in the same manner as the experimental cultures.

Thus the experimental and control cultures were grown from inocula of comparable size in a medium containing both acriflavine and nucleate. In the case of the experimental cultures, the inoculum consisted of survivors of a prior exposure to acriflavine alone, whereas in the controls untreated bacteria were used.

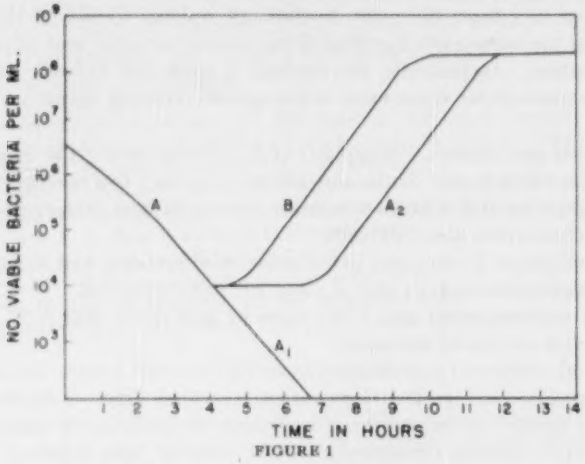


FIGURE 1

Killing and Growth in Experimental and Control Cultures. $A$-$A_1$ = killing curve of B/r in 0.01% acriflavine in broth; $A_2$ = growth of acriflavine-treated bacteria after addition of 0.5% sodium nucleate (experimental cultures); $B$ = growth of untreated bacteria in broth containing 0.01% acriflavine + 0.5% sodium nucleate (controls).

*Experimental Results.—Growth in Nucleate-Inactivated Acriflavine:* Figure 1 shows the course of killing and growth in the experimental cultures, and of growth in the controls. Killing proceeds logarithmically in acriflavine until the addition of nucleate, at which time it is sharply arrested. The growth of the survivors in the nucleate-acriflavine mixture is comparable to that of the controls, except for the prolongation of the lag phase. The final titers of experimental and control cultures were not significantly different.

*Frequency Distribution of Phage-Resistant Mutants in Experimental and Control Cultures.*—Figure 2 shows the frequency distribution of mutants resistant to bacteriophage T1 in 300 experimental cultures and 300 control cultures. The spread observed in the control series is a function of the spontaneous mutation rate and the clonal grouping of mutants in liquid cultures,[5] and has been found to be extremely reproducible. It will be seen that the distribution of mutants in the experimental cultures is very different. The largest class of cultures in the experimental series is that having between $10^2$ and $10^3$ mutants per $10^8$ bacteria, whereas the largest control class has less than 10 mutants per $10^8$ bacteria. The frequency distributions for both experimental and control culture series are highly

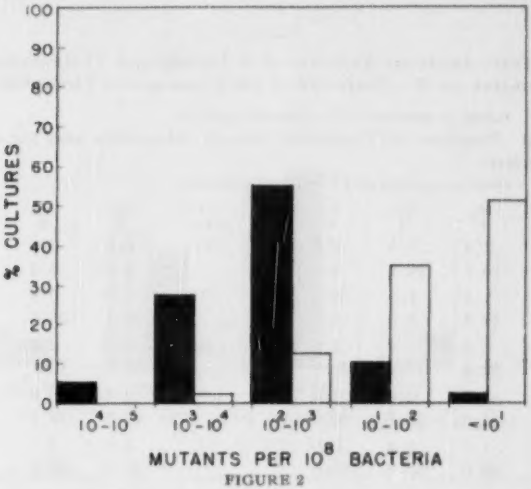


FIGURE 2

Frequency distribution of T1-resistant mutants for 300 experimental cultures (black columns) and 300 control cultures (white columns).

reproducible under the conditions specified, as will be shown below in another connection.

*Selection.*—The observed shift in the frequency distribution of mutants in experimental cultures can be accounted for by induced mutation only if two possible modes of selection can be ruled out. Selection could enter through differential survival of spontaneous mutants in the experimental cultures during exposure to acriflavine, or through differential stimulation of the rate of growth of phage-resistant mutants by the acriflavine-nucleate mixture, or through a combination of the two. These possibilities were tested in the following way: Ten single-colony isolates of phage-resistant

mutants were made, each colony coming from a plating on phage of a different experimental culture. If selection were responsible for the increased frequency of mutants in these cultures, mutants thus obtained should possess the particular advantages involved, having been through the selective sieve of the experimental procedure. After repeated streaking and single-colony isolation to eliminate contaminating phage particles, the ten phage-resistant strains were grown in broth. Mixtures were made of each mutant strain with the phage-sensitive parent strain, and each mixture was assayed to determine the relative proportions of its components. The mixtures were then subjected to the experimental procedure described above: inoculation into acriflavine, and incubation for four hours followed by addition of sodium nucleate and incubation for 18-24 hours. Assays to deter-

TABLE 1

SELECTION TEST—ARTIFICIAL MIXTURES OF 10 INDEPENDENT T1-RESISTANT MUTANT STRAINS AND B/r, SUBJECTED TO THE EXPERIMENTAL PROCEDURE

$M_o$, per cent Initial proportion of T1-resistant mutants
$M_a$, per cent Proportion of T1-resistant mutants immediately after the addition of sodium nucleate
$M_f$, per cent Final proportion of T1-resistant mutants

| B/r/1 STRAIN | $M_o$, % | $M_a$, % | $M_f$, % | B/r/1 STRAIN | $M_o$, % | $M_a$, % | $M_f$, % |
|---|---|---|---|---|---|---|---|
| 1 | 1.3 | 1.9 | 0.6 | 6 | 0.6 | 0.8 | 0.3 |
| | 15.3 | 18.1 | 9.4 | | 8.6 | 6.2 | 4.1 |
| 2 | 1.1 | 1.3 | 0.7 | 7 | 0.9 | 0.8 | 0.4 |
| | 10.3 | 8.6 | 9.1 | | 23.1 | 32.6 | 19.3 |
| 3 | 1.4 | 1.4 | 1.5 | 8 | 1.5 | 2.0 | 1.8 |
| | 22.4 | 17.4 | 20.2 | | 13.7 | 10.1 | 15.4 |
| 4 | 0.7 | 0.9 | 1.0 | 9 | 0.5 | 0.8 | 0.8 |
| | 14.5 | 10.7 | 20.1 | | 19.3 | 29.2 | 14.6 |
| 5 | 1.1 | 0.9 | 0.8 | 10 | 3.0 | 2.1 | 1.8 |
| | 46.0 | 33.2 | 35.6 | | 36.8 | 56.6 | 42.0 |

mine the proportions of phage-resistant and phage-sensitive components were made immediately after the addition of nucleate, and again after the 18- to 24-hour incubation period. The results are shown in table 1. It is evident that selection does not favor the phage-resistant mutants, either by differential survival or by differential growth.

*Mutagenic Effect of Acriflavine on an Acriflavine-Resistant Strain.*—The growth of the normal B/r strain is completely inhibited by a concentration of 0.002% of neutral acriflavine in broth. By serial transfer in increasing concentrations of acriflavine, a strain was derived from B/r which could grow in 30 times this concentration, or 0.06%. This acriflavine-resistant strain, when treated with acriflavine according to the standard procedure described above, using a concentration of 0.01% acriflavine, gave a frequency distribution of phage-resistant mutants identical with

that of the untreated controls. The four-hour exposure to this concentration of acriflavine was entirely non-toxic for the resistant strain. When the experiment was repeated, however, using a concentration of 0.3%, which gave a survival of $10^{-2}$ to $10^{-3}$, the frequency distribution of phage-resistant mutants was essentially the same as that obtained with strain B/r at the 0.01% concentration, which yields the same survival during the 4-hour treatment. Figure 3 shows the frequency distributions of phage-resistant mutants obtained for the acriflavine-resistant strain, using 0.3% acriflavine, as compared with those obtained for B/r at a concentration of 0.01%, and for untreated controls. It is evident that the mutagenic effect

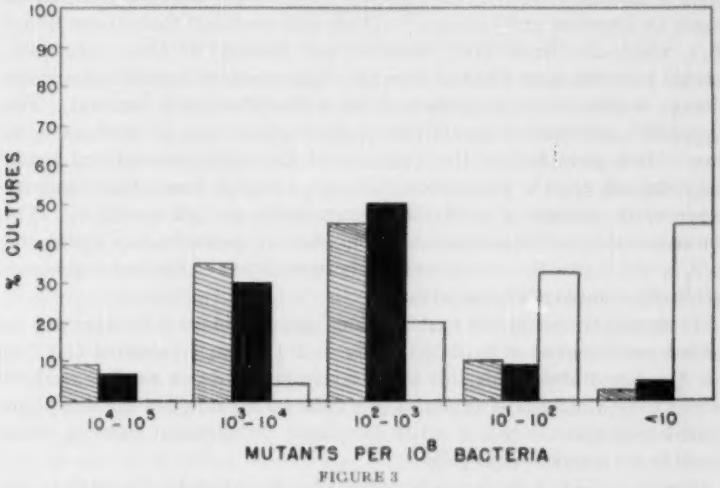


FIGURE 3

Frequency distribution of T1-resistant mutants for 100 experimental cultures of acriflavine-resistant bacteria (cross-hatched columns); 100 experimental cultures of acriflavine-sensitive bacteria (black columns); and 100 controls (white columns).

of acriflavine is related to the survival levels of the sensitive and resistant strains, rather than to the absolute concentration of acriflavine used in the treatment.

*Discussion.*—The mutagenicity of acriflavine is confirmed in these experiments, under conditions free of the complications encountered earlier and enumerated above. The method is essentially qualitative, since the cultivation following the acriflavine treatment introduces clonal variance, which makes precise determination of the frequency of induced mutations extremely difficult. In addition, the method as described does not permit distinction between "zero-point" and delayed mutations, and both types could contribute to the greater frequency of cultures having high numbers

of mutants. Modifications that would permit the separation of the two types of induced mutations are theoretically possible, however. One such procedure would involve adding bacteriophage to the nucleate-inactivated acriflavine tubes immediately after adding nucleate, so that only tubes containing at least one zero-point mutant would develop turbidity. Addition of bacteriophage after various incubation periods would similarly permit the measurement of delayed mutations. Preliminary experiments along these lines have been done, with promising results.

The observation that the acriflavine-resistant strain requires a much higher concentration of acriflavine than the original strain to produce a given level of mutagenic activity is of interest in relation to a similar comparison made by Demerec and Latarjet.[6] These authors found that strains B and B/r, which are, respectively, sensitive and resistant to ultra-violet radiation,[7] give the same yield of induced phage-resistant mutants at a given dosage of ultra-violet, regardless of the wide difference in survival. The apparent contradiction between these observations may be spurious, however. It is possible that the resistance of the acriflavine-resistant strain may depend upon a permeability change, or upon some other property whereby the amount of acriflavine present inside the cell is reduced. The survival level may be a measure of the effective concentration within the cell, in which case the correspondence between levels of survival and mutagenic effect would not be surprising.

It should be mentioned that in using this procedure it is necessary to adjust conditions so as to obtain survivals in the range indicated ($10^{-2}$ to $10^{-3}$). Survivals outside this range rarely give positive results, as there seems to be a delicate interplay between the killing rate and the rate of induced mutation. For any other compound, the optimal survival range must be determined empirically.

*Summary.*—(1) A method is described whereby exposure of bacteria to the action of acriflavine can be sharply arrested, and its residual activity eliminated by the addition of sodium nucleate.

(2) The mutagenic activity of acriflavine, as measured by its ability to induce mutations from phage-sensitivity to phage-resistance in strain B/r of *E. coli*, is confirmed by the use of the nucleate-reversal technique.

(3) An acriflavine-resistant strain of *E. coli*, derived from B/r, requires 30 times as much acriflavine as does B/r to give a comparable mutagenic effect, as well as a comparable level of survival.

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## THE NATURE OF BONE AND PHOSPHATE ROCK

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Carbonate has never been adequately explained as a constituent of bone, dentine, enamel, and the closely related phosphorites. Its failure to act quite like $CaCO_3$, upon heating for instance, has, since the time of Hoppe-Seyler[1] led to postulated calcium carbonate phosphate compounds as essential constituents of bone or enamel. Existence of optically homogenous apatites such as the minerals francolite[2] and collophane containing carbonate apparently supports this conclusion and has led to the acceptance of an apatite structure containing carbonate.

Preferential solution by acid of carbonate in bone has often been noted[3–5] and has been interpreted by some workers[4] as evidence for the presence of $CaCO_3$ as a separate phase. Crystalline $CaCO_3$, however, has not been observed in bone by the very sensitive test of its high birefringence. A Geiger-counter x-ray spectrometer, moreover, gives no evidence of its presence, while one-third as much calcite as would correspond to the analysis can be detected when mixed with hydroxylapatite, or naphtha-extracted bone. More incontrovertible evidence is afforded by the work of Logan and Taylor[5] which shows that an apatite phase will precipitate, from a solution having the inorganic composition of plasma, in the presence of carbonate and steadily withdraw $CO_3^{--}$ from a solution unsaturated with respect to $CaCO_3$.

Bone, enamel, dentine and collophane, the last being the apparently isotropic component of phosphate rocks, give rather diffuse x-ray powder diffraction patterns identical, within their definition, with that of fluor-apatite.[6] Francolite, the more highly birefringent material of phosphorites, can be obtained as optically clear single crystals containing more than 3% $CO_2$.[2] X-ray goniometer photographs of francolite crystals are identical with those of fluorapatite, even in very high orders of interference maxima. From the structural point of view it seemed necessary for a large number of $CO_3$ groups to be present in an apatite structure without changing the diffraction pattern, even in minor ways, which is very unlikely.

The average inorganic compositions of human bone, and enamel, based upon a great number of published analyses, can be represented by the following formulas[7] in which the number of positive ions is taken as 10.0:

Bone $[Ca_{9.86}Mg_{0.23}Na_{0.22}][(PO_4)_{5.66}(CO_3)_{1.38}][H_2O]_x$

Enamel $[Ca_{9.79}Mg_{0.19}Na_{0.11}][(PO_4)_{5.89}(CO_3)_{0.48}][OH]_{1.57}[H_2O]_x$

Three quite different suggestions for the structural nature of the compound present have been advanced in recent years. Trömel and Möller[8] and Bale and coworkers[6, 9] consider the material to be hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, with "adsorbed" carbonate and possibly phosphate. Bale[6] states "Since the diffraction pattern of tooth and bone substance give no indication of a carbonate apatite being present, the carbonate ions are also probably most abundant in these regions of structural discontinuity that may be considered as marking the transition from one crystalline particle to another." Brasseur and Dallemagne in a series of papers published in Belgium during the war which were summarized in *Nature* in 1946[10] (also reference 4) concluded that bone is "tricalcium phosphate hydrate," $Ca_9(PO_4)_6(H_2O)_2$, with $CaCO_3$ present as a separate phase and that enamel is in part "carbonate hydroxylapatite." They state "We cannot admit, therefore, that the tertiary with a Ca/P ratio of 1.94 is hydroxylapatite with adsorbed $PO_4$ ions." Hendricks and Hill in 1942[7] reached essentially these same conclusions except that they considered the carbonate of bone to be present in the "tricalcium phosphate hydrate" lattice.

Properties of the several materials pertinent, without further discussion, to the question at issue are:

1. A single phase having the x-ray diffraction pattern of apatite and containing carbonate is present even though bone forms in a system unsaturated with respect to calcium carbonate.

2. In human bone this phase is shown by analysis to be a neutral substance. It is more basic, approaching hydroxylapatite, in parts of enamel.

3. A large portion of the carbonate in bone can be preferentially removed by solution in acid without destruction of the apatite lattice. Enamel and some "carbonate apatites" dissolve uniformly.

4. Citrate is present in bone as water insoluble form in amounts by volume approaching one-half that of carbonate.[11, 12]

The first and third of these properties indicate rather clearly that carbonate is on the surface of the bone material. Presence of citrate can also be explained in this way instead of being merely "adventitious." Bone also contains magnesium and sodium that might be expected to replace $Ca^{++}$ in the apatite structure on the basis of ionic radii for $Na^+$ and $Mg^{++}$. These two are preferentially dissolved by acid,[8] however, indicating their location on the lattice surface. The difficulties of charge with

$Na^+$ replacing $Ca^{++}$ and essential absence in solutions near neutrality of magnesium as $Mg^{++}$, rather being $Mg(OH)^+$, accordingly do not arise. There is still a size limitation for the surface, however, since $Na^+$ is taken up preferential to $K^+$.

The nature of "carbonate apatites" has been most confusing in its bearing on the type of compound present in bone. Lack of preferential solution in acid of carbonate in enamel was essentially the reason for Brasseur and Dallemagne considering the compound to be "carbonate hydroxylapatite." Another possibility, however, is that the carbonate groups of enamel and francolite and the citrate of enamel are also present

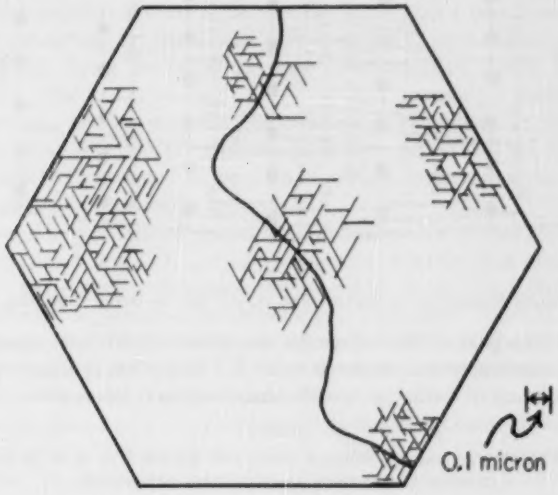


FIGURE 1

Entrapped surfaces in "carbonate apatites" as present in enamel and francolite, schematic.

on surfaces which are entrapped as crystallites grow and are surrounded by phosphate such that the final material has many occluded surfaces as schematically shown in figure 1. In the case of francolite, and to a lesser extent in enamel, the apatite lattice can be continuous throughout large crystals. The high density of francolite[2] indicates that voids cannot constitute more than 1% of the total volume.

Evidence in regard to entrapped surfaces for francolite and collophane comes from some peculiarities of their fluorine contents which have not previously been explained. These substances[2, 13] contain fluorine in excess of that required for an apatite structure, sometimes by a factor as great as

1.50, while fluorapatite has not been found to contain excess fluorine. Now if the surface occurs at primitive translations and all fluorine positions

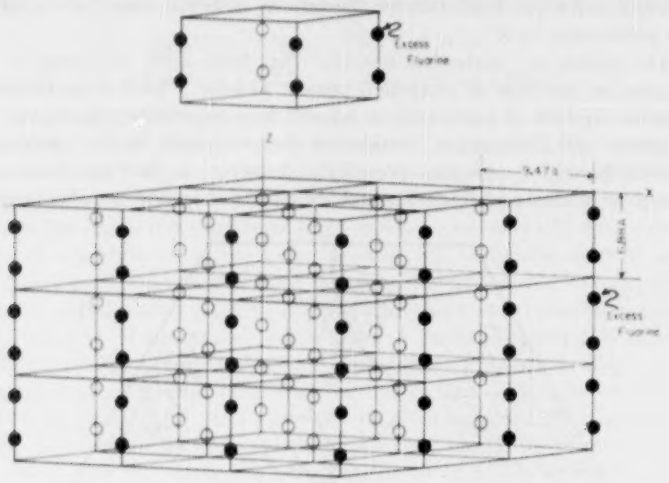


FIGURE 2

Excess fluorine in an apatite-type crystal due to lattice limitation.

are filled then part of the surface fluorine atoms would be in excess of the unit cell requirements as shown in figure 2. Francolite is always observed to be secondary in formation to collophane and as it forms with a decrease

TABLE 1

EFFECT OF FRAGMENT SIZE ON SURFACE AREA AND SOLUTION IN ACID OF SOME CARBONATE CONTAINING APATITE TYPE SUBSTANCES

| SUBSTANCE | FINENESS SIEVE SIZE | SURFACE, M.²/G. | PARTIAL SOLUBILITY IN ACID, PER CENT OF TOTAL[6] | | |
|---|---|---|---|---|---|
| | | | $CO_2$ | CaO | $P_2O_5$ |
| Steamed bone | 100–200 | 66.2 | 18.6 | 6.5 | 6.5 |
| | 16– 30 | 66.2 | 15.5 | 5.9 | 5.4 |
| | 4– 8 | 63.7 | .. | ... | ... |
| Francolite | Below 6 micron | 7.3 | 26.1 | 7.2 | 2.9 |
| | 100–200 | 3.2 | 10.5 | 6.6 | 3.9 |
| | 20– 35 | 1.6 | 6.5 | 3.7 | 3.5 |
| Enamel | Through 325 | 6.8 | | | |
| | 200–325 | 3.7 | | | |
| | 120–200 | 3.2 | | | |
| | 60–120 | 2.1 | | | |

[6] Analyses by J. H. Caro.

in surface, excess fluorine present on the surfaces of primary collophane is exuded in limited amounts as secondary fluorite[14] in many phosphorites.

Other evidence for entrapped surfaces can be obtained from surface area measurements by nitrogen absorption (B.E.T.). These areas should depend markedly upon screen mesh size of material under test if entrapped surfaces are uncovered by breakage as indicated in figure 1. That this is true for enamel and francolite, but markedly less so for bone as required by the preferential solution of its carbonate in acid is shown by results in table 1. These surface areas were measured in the laboratory of Dr. V. R. Dietz of the National Bureau of Standards who, independently, had made the observations on enamel for Dr. R. C. Likens of the National Institutes of Health.

Attention is now turned to some detailed consideration of the surfaces. Bone, after removal of most of its organic matter, has a maximum surface of about 80–100 m.²/g. (Reference 15 and extensive unpublished information of V. R. Dietz for bone ash) and contains about $7.5 \times 10^{20}$ $CO_3$ groups/g.[7] The corresponding area per group, assuming a surface area of 100 m.²/g., the larger value being taken in allowance for entrapped surfaces, is 13.3 A² which is about the value to be expected if the carbonate (and possibly citrate ions) forms a single surface layer containing cations and the maximum surface is realized.

Now consider the francolite sample analyzed by Gruner and McConnell[2] which contains 3.36% $CO_2$ and 1.08 times more fluorine than required for apatite. The excess surface fluorine is given by $2x + 1 = 0.08x^2$, where $x$ is the number of primitive translations in a "crystallite" which is assumed to be equal in the $x$ and $y$ directions, figure 2. Thus $x$ is about 25 corresponding to 234 A ($25 \times 9.35$ A). The calculated "surface area"/g., made up chiefly of that entrapped, is about 62 m.²/g. The "external surface" is surely less than 1 m.²/g. A completely independent estimate of surface area is afforded by the carbonate content. For 3.36% $CO_2$ and an area of 13.3 A² per $CO_3$ group this is 61 m.²/g. While the agreement with the other value is perfect the combination of the methods can only be trusted to about 25%, the greatest uncertainty being about the surface area per $CO_3$ group.

The nature of "tricalcium phosphate hydrate" having an apatite diffraction pattern, and serving as a prototype for bone, is now considered. This material can be formed by precipitating calcium phosphate in a system having a pH near that of plasma or by hydrolysis of $CaHPO_4$, i.e., under conditions where $HPO_4^{--}$ is a predominant phosphate ion in solution. The composition of this material determined by independent groups is $Ca_9(PO_4)_6(H_2O)_{2.7}$ or $Ca_9(PO_4)_6(H_2O)_{1.5}$,[16] the question of the exact water content of course being difficult to settle. A preparation made by slow hydrolysis of $CaHPO_4$ in a system more acid than pH 5.0 contained crystals measuring about 2 microns in length which were shown by electron micrographs to be well formed lath-like prisms with pyramidal faces. A ma-

terial of this type gradually lost 4.70% $H_2O$ (calculated for $Ca_9(PO_4)_6(H_2O)_2$ 3.73% $H_2O$) between 100° and 800°. Above 800° the substance is essentially anhydrous and gives the diffraction pattern of the beta modification of $Ca_3(PO_4)_2$ (stable below 1115°C. and possibly a stable phase in solution) which verifies the molal ratio. Hydroxylapatite behaves in a markedly different way in retaining its full content of 1.79% $H_2O$ upon heating at 1000°. It is dehydrated fully only above 1300°.

Is this substance really what it appears to be or is it hydroxylapatite as was suggested many years ago by Trömel[8] (also references 6 and 9) with surface material modifying the composition? The "formula" on this basis would be

$$Ca_{10}(PO_4)_6(OH)_2\text{—}\begin{matrix}\text{Surface } (PO_4)_{0.67} \\ \text{Surface } (H^+)_{2.00}\end{matrix}$$

and would have to be approximated, on the basis of present evidence, over a pH range from about 4.6 to 7.3 in the precipitating system. The particular composition might vary somewhat and would be determined possibly by the surface and the pH. Surface areas of several materials made by precipitation varied from 26 to 64 $m.^2/g.$ With surfaces, separated by perhaps ten primitive translations and entrapped in part, covered with $HPO_4^{--}$ ions, behavior upon heating might parallel that observed; namely, gradual loss of water in a manner very different from that of hydroxylapatite. In the case of bone, $HPO_4^{--}$ would have to be present on the surface together with carbonate and citrate. Experimental results at hand cannot safely resolve the question, although the authors, as well as Brasseur and Dallemagne, have previously considered the evidence adequate for "tricalcium phosphate hydrate." The observation that the poorly developed x-ray powder diffraction pattern is closely the same as that of hydroxyl apatite should surely be given full weight,[6] but it alone is not conclusive.

This surface chemistry of bone and related materials markedly affects composition and is a result or probable cause of the large surface area. It can be generalized. An insoluble calcium compound can form surface compounds with other ions having insoluble or poorly ionized calcium salts (e.g., citrate). Thus $CaCO_3$ would equally be expected to form surface phosphates and citrates in much the same way as calcium phosphates form surface carbonates. Such reactions which are important for phosphate fertility of calcareous soils have been observed in tracer experiments by Dr. Sterling Olson of this Bureau.

The phenomenon discussed here, which can be studied in many ways, has broad implications in the formation and properties of bone and teeth. An explanation is afforded for the unusual relationship of fluorite, collophane and francolite in phosphate rock. Interpretation can be given to

occurrence of uranium and other groups as surface material in phosphorites.[17] An understanding is afforded for the preferential use of certain phosphate rocks, particularly those from North Africa having high carbonate contents, for direct application to some crops.[18]

Modification of composition by small particle size has also been found for hydroxylapatite by P. W. Arnold of Cambridge University (personal communication). He observed that precipitates of hydroxylapatite contain $(OH)^-$ in excess of the formula requirements, such as to yield more than 2.0% water between 900° and 1400°C. (calculated 1.79% $H_2O$).

*Summary.*—The inorganic compounds of bone have a large surface on which carbonate, citrate, magnesium and sodium are located. Carbonate is present on entrapped surfaces and not as constituent within the lattice of the mineral francolite, the typical "carbonate apatite," that also by lattice limitation, contains fluorine in excess of the fluorapatite requirement. Enamel is similar to francolite in having entrapped surfaces. The over-all composition, including lattice and surface, of bone is that of a neutral calcium phosphate while enamel is more basic, approximating hydroxylapatite. The compound forming the lattice of bone remains to be established but it might well be hydroxylapatite with an excess of phosphate on the surface.

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# LIMITATION OF ELECTRON DENSITY BY THE PATTERSON FUNCTION

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Communicated by W. J. Mead, November 1, 1950

In several recent papers[1-3] it has been shown that the vector set can be solved for its fundamental set, and this implies, in a broad way, that a crystal structure can be solved from the intensities of its x-ray diffraction spectra. In particular, it implies that hitherto unsuspected relations exist between the Patterson function and the electron density function.

One such relation has been demonstrated: If an image of any polygon, whose $n$ vertices ($n \geqslant 2$) have coordinates $x_1y_1z_1$, $x_2y_2z_2$, ..., $x_ny_nz_n$, can be found, one can construct the function

$$\Pi_n(xyz) = P(x_1y_1z_1) \times P(x_2y_2z_2) \times \ldots, P(x_ny_nz_n), \qquad (1)$$

where $P(x_1y_1z_1)$, etc., are Patterson functions. Then if there are no equal vectors between points in the fundamental set, there are no fortuitously coincident images in the vector set, and the following relation holds,[3] except for an exaggeration due to the selected image point:

$$\rho(xyz) = K \sqrt[n]{\Pi_n(xyz)}, \qquad (2)$$

where $K$ is a constant defined by the chosen image.

For actual crystals, the electron density varies continuously with the coordinates, so that the Patterson synthesis contains a continuous overlapping of images. These arise not only from Patterson vectors radiating from a particular origin, but also from Patterson vectors reaching into a particular cell from the origins of the neighboring cells. The Patterson function for an actual crystal therefore contains extensively overlapped and coincident images. This feature always degrades the information obtainable from (1) in a specific way, namely by increasing the values of one or more $P$'s and therefore increasing the value of $\Pi$. For an actual crystal, therefore, an inequality corresponding to (2) holds, namely

$$\rho(xyz) \leqslant K \sqrt[n]{\Pi_n(xyz)}. \qquad (3)$$

In spite of this unfortunate feature, the $\Pi$ function is useful in the interpretation of Patterson maps. Only high ground can be occupied by atoms, and low ground can be regarded as unpermitted territory. In instances where the overlapping is minimized, the function is most useful. For example, a collection of heavy atoms embedded in a matrix of lighter ones can be readily located by the $\Pi$ function. The reason for its power in such an instance is that the characteristics of the set of heavy atoms

approaches that of a set of pure points reasonably separated in empty space.

A particularly bad feature of the II function depends on the fact that a Patterson map usually has a more or less continuous non-zero background which arises from at least partial overlapping of the images of atoms. When a line image, for example, encounters a multiple peak at one end, it usually has this background at its other end, so that the product has some moderate value. Because of this, the II function often produces a ghost when one point of the polygon passes over a large Patterson peak, in spite of the fact that the polygon location is not an image position. Due to the presence of such ghosts, the II function usually has more peaks than the electron density function.

FIGURE 1
Centrosymmetrical fundamental set.

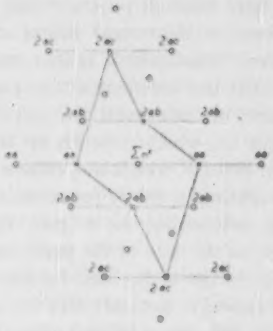

FIGURE 2
Vector set of the fundamental set of Fig. 1, showing weighting of points (circles) and also values of the $M_2$ function (crosses).

Obviously, the II function is not the only image-seeking function which can be devised to locate atoms. Any function which drops to zero when the weighting on at least one of the polygon vertices is zero can be expected to map the electron density with more or less faithfulness. A particular function which avoids the disadvantage of the II function mentioned in the last paragraph may be called an $M$ function. To form the $M_n$ function, one records at $xyz$ the minimum value of the $n$ values of the Patterson function which occur at the $n$ vertices of the roving image polygon. It will be demonstrated that this function has the interesting property that

$$\rho(xyz) \leqslant KM_n(xyz), \tag{4}$$

using the same notation as for (2). Inequality (4) is more powerful than (3).

The general properties of the $M$ function can be readily appreciated if it is applied to the case of a polygon image of $n$ points which are related by symmetry in the crystal. For simplicity and clearness, consider $n = 2$, that is, a line image, and apply this image to a vector point-set. Let the point at the end of the origin line-image be a product ("interaction") between centrosymmetrically related points in the crystal. This is illustrated in figures 1 and 2. Figure 1 shows the points in a cell of the crystal and their weights; figure 2 shows the vector points arising from the points within the single cell shown in figure 1. The dotted lines of figure 2 show the line images, and the cross on each one shows the location of a peak of the $M_2$ function. The labeling indicates the weights of the peaks. Incidentally, note that all points of the vector set which are "interactions" between centrosymmetrical points of the crystal set have unit weight, while other "interaction" points have double weight. It will also be observed that the locations of the peaks of the $M_2$ function are the same as the points of the crystal set, and their magnitudes are the same except for a factor $2a$, which depends on the weight (corresponding to electron density) of point $a$, which was chosen for the first image point. An exception in weighting in the $M$ function occurs for the point reproduced by the line image containing the origin; this has unit weight. The exception occurs only in the case of the point associated with the vector peak chosen for the line image to be used for the decomposition of the vector map.

The $M$ function not only has the advantage mentioned above over the $\Pi$ function, but also a related advantage of usually giving a more correct value when the image polygon comes to an image location for which one of the vertices lies on a multiple peak. As the $\Pi$ function ranges over such an overlapped peak, it lays down an exaggerated peak, but the $M$ function lays down a peak of normal magnitude provided that at least one vertex of the image polygon is not overlapped. Under such conditions, therefore (except for the neighborhood of the atom $a$ used as the initial image points) the $M$ function has the important property

$$\rho(xyz) = \frac{1}{2\rho_a} M(xyz). \tag{5}$$

In the case of an actual crystal, the fundamental set becomes the electron density map, and the vector set becomes the Patterson map.[3] There is usually overlapping to some extent at all vertices of the image polygon in the Patterson map. But even when this occurs, it merely increases the value of $M$, and the inequality

$$\rho(xyz) \leqslant \frac{1}{2\rho_o} M(xyz) \qquad (6)$$

is valid. Relation (6) implies that the Patterson function places an upper allowable limit of the electron density at every point in the cell. Since the electron density at the center of an atom is a characteristic of an atom, this relation will permit locating atoms in instances where the Patterson is not complicated by too much overlapping. In any case, it permits eliminating regions where a specific atom cannot be located, on the ground that the electron density at the atom center is greater than that of the $M$ function in the region.

In crystals having symmetry other than an inversion center, a centrosymmetrical fraction of the cell is repeated by other symmetry elements to form the entire cell. When the image polygon is chosen so that it does not conform to the Patterson symmetry at the origin, it does not, in general, encounter symmetrical situations at equivalent locations in these fractions of the cell. Nevertheless, if the Patterson map contains no fortuitous overlaps, any image function synthesizes the true symmetry of the crystal. But if there are fortuitous overlaps, the image function may synthesize a degradation of the true symmetry. For example, consider a two-dimensional Patterson map of symmetry *p2mm*, and let a line image be chosen which, at the origin, makes an oblique angle with both symmetry lines. In reproducing symmetrically located peaks on the two sides of a symmetry line, the sloping image line uses two peaks on one side of the symmetry line and two peaks on the other side of the line, respectively. These two pairs of peaks are not symmetrically related unless the line image conforms to the symmetry of the Patterson map. Since, in general, each pair of peaks is involved in different overlaps, any line function reproduces the symmetry of the crystal as degraded by the overlap characteristic of the cell fraction. In such instances, the minimum value of either the II or the $M$ function at equivalent points in different cell fractions can be combined to give a better minimum value of II or $M$, thus increasing the power of the particular inequality (3) or (6).

Since (6) is quantitative, it requires the Patterson synthesis upon which it is based to be quantitative. Any feature which reduces the accuracy of the Patterson synthesis therefore reduces the effectiveness of (6). A particularly bad effect has been noticed which is caused by the limited number of experimental $F^2$'s available and the consequent termination of the Fourier series used for the synthesis of the Patterson map. This gives rise to false sets of undulations corresponding to optical diffraction fringes. A set of such undulations surrounds each strong Patterson peak, but the undulations are most noticeable surrounding the great origin peak. The ring-shaped trough and the following ring-shaped hill which immediately surround the origin are very objectionable and tend to distort

the value of the $M$ function as the image polygon passes over them. It is recommended that before computing an $M$ function from Patterson data these undulations be eliminated along the lines suggested by James.[4]

As described above, the $M$ function was applied to polygon images composed of points due to symmetrical "interactions." It can also be applied to image polygons containing points due to non-symmetrical "interactions." The selected image is then a polygon whose $n$ points have weights, say, $aa, ab, ac, \ldots$. To form the $M$ function for this polygon, one first prepares $n$ Patterson maps of the sampling of the Patterson function scaled, respectively, by factors $1/a$, $1/b$, $1/c$, ..., and lets one point of the image polygon rove over the corresponding map. When the polygon is located at $xyz$, the minimum value which occurs at the $n$ points is then recorded at $xyz$, and the following relation holds

$$\rho(xyz) \leqslant M(xyz). \tag{7}$$

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## *SOME LINEAR OPERATORS IN THE THEORY OF PARTIAL DIFFERENTIAL EQUATIONS**

BY S. BERGMAN AND M. SCHIFFER

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Communicated by Marston Morse, September 29, 1950

1. Let $D_2$ be a finite domain in the $x$, $y$-plane bounded by an analytic curve $C_2$. Let $D_1$ be a proper subdomain of $D_2$ with analytic boundary curve $C_1$. Denote finally the difference domain bounded by $C_1$ and $C_2$ by $D_0$.

Consider the partial differential equation of elliptic type

$$\Delta u = q(P)u, \qquad \Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \tag{1}$$

where $q(P)$ is a positive continuously differentiable function of the point $P \equiv (x, y)$ in $D_2 + C_2$. We introduce the scalar products

$$E_i\{u, v\} = \iint_{D_i} [\operatorname{grad} u \cdot \operatorname{grad} v + quv]d\tau, \qquad i = 0, 1, 2, \tag{2}$$

$d\tau$ = area element in $D_t$

and the three Hilbert spaces $\Sigma_t$ consisting of all solutions $u$ of (1) with a finite norm $E_t(u) = E_t\{u, u\}$. Let finally $N_t(P, Q)$ and $G_t(P, Q)$ denote Neumann's and Green's functions with respect to the differential equation (1) and the domain $D_t$.

We define the kernels

$$K_t(P, Q) = N_t(P, Q) - G_t(P, Q), \; L_t(P, Q) = N_t(P, Q) + G_t(P, Q) \tag{3}$$

which play an important role in the theory of the spaces $\Sigma_t$ [1, $a - d$; 2] and each of which is defined in the domain $D_t$. We may construct now discontinuous solutions of (1) which are defined in $D_2$, composed in a simple way of the kernels (3) and possess various interesting properties. We set

$$l(P, Q) = \begin{cases} L_1(P, Q) - L_2(P, Q) & \text{for } P, Q \in D_1 \\ - L_2(P, Q) & \text{for } P \in D_1, Q \in D_0 \text{ or } P \in D_0, Q \in D_1 \\ L_0(P, Q) - L_2(P, Q) & \text{for } P, Q \in D_0. \end{cases} \tag{4}$$

Similarly, we define

$$k(P, Q) = \begin{cases} K_1(P, Q) - K_2(P, Q) & \text{for } P, Q \in D_1 \\ - K_2(P, Q) & \text{for } P \in D_1, Q \in D_0 \text{ or } P \in D_0, Q \in D_1 \\ K_0(P, Q) - K_2(P, Q) & \text{for } P, Q \in D_0 \end{cases} \tag{5}$$

Using the characteristic properties of the various Green's and Neumann's functions, the following identity can be established:

$$E_2\{l(P, Q), l(P, R)\} = k(Q, R), \qquad Q, R \in D_2 \text{ but not on } C_1 \tag{6}$$

Here the convention will be used that if kernels with various argument points are multiplied the operation shall always be understood with respect to the common argument letter. Similarly, we can show

$$E_2\{l(P, Q), k(P, R)\} = l(Q, R), \qquad Q, R \in D_2 \text{ but not on } C_1, \tag{6'}$$

and

$$E_2\{k(P, Q), k(P, R)\} = k(Q, R), \qquad Q, R \in D_2 \text{ but not on } C_1 \tag{6''}$$

2. Let $\Sigma$ denote the Hilbert space of all functions in $D_2$ which represent in $D_0$ an element of $\Sigma_0$ and in $D_1$ an element of $\Sigma_1$. The metric in $\Sigma$ will be based on the scalar product $E_2\{u, v\}$.

We define in $\Sigma$ the linear operators [cf. references 1*d* and 3]

$$T_u(P) = E_2\{l(P, Q), u(Q)\}, \qquad S_u(P) = E_2\{K_2(P, Q), u(Q)\}, \tag{7}$$

which transform the space $\Sigma$ into itself. They satisfy the following relations:

$$E_2\{u, v\} = E_2\{T_u, T_v\} + E_2\{S_u, S_v\} \tag{8}$$

$$E_2\{T_u, S_v\} = 0. \tag{8'}$$

These results suggest the introduction of the two linear operators in $\Sigma$:

$$\mathfrak{S}_u^+ = T_u + S_u \qquad \mathfrak{S}_u^- = T_u - S_u \tag{9}$$

which have the property of preserving the metric

$$E_2\{\mathfrak{S}_u^+, \mathfrak{S}_v^+\} = E_2\{u, v\}, \qquad E_2\{\mathfrak{S}_u^-, \mathfrak{S}_v^-\} = E_2\{u, v\}. \tag{10}$$

The operators $\mathfrak{S}_u^+$ and $\mathfrak{S}_u^-$ have further the following involutory character:

*If* $v = \mathfrak{S}_u^+$, *then* $u = \mathfrak{S}_v^+$ *and if* $w = \mathfrak{S}_u^-$ *then* $u = \mathfrak{S}_w^-$. This result shows that $\mathfrak{S}^+$ and $\mathfrak{S}^-$ are one-one mappings of $\Sigma$ onto itself.

Each function $u \in \Sigma$ can be decomposed in a unique way into

$$u(P) = a(P) + b(P) \tag{11}$$

where $b(P) \in \Sigma_2$ and $a(P)$ is orthogonal to $\Sigma_2$. The operators $T$ and $S$ perform this orthogonal decomposition of $\Sigma$.

The operators $\mathfrak{S}^+, \mathfrak{S}^-$ can easily be interpreted as follows: Given an element $u \in \Sigma$, which is continuously differentiable in the closures of $D_0$ and $D_1$, $\mathfrak{S}_u^+$ will be that function of $\Sigma$ which has on $C_2$ the same boundary values as $u$, which has along $C_1$ the same discontinuity a s$u$ but whose normal derivative has the discontinuity of opposite sign on $C_1$ than has $\frac{\partial u}{\partial \nu}$.

Similarly $\mathfrak{S}_u^-$ has on $C_2$ the same normal derivative as $u$, its normal derivative has the same jump through $C_1$ as that of $u$, but its discontinuity across $C_1$ is at each point minus the jump of $u$ over that line.

3. Let $u \in \Sigma$ be identically zero in $D_0$. In this case $E_2(u) = E_1(u)$ and we derive from (8):

$$E_1(u) \geqslant E_1(T_u). \tag{12}$$

This inequality holds for every function $u \in \Sigma_1$. We derive from it, in particular, that the eigenvalues of the integro-differential equation

$$u_\rho(P) = \lambda_\rho E_1\{l(P, Q), u_\rho(Q)\}, \qquad Q \in D_1 \tag{13}$$

are all greater or equal to 1 in absolute value. A finer study of the conditions for the equality sign shows that always

$$|\lambda_\rho| > 1 \tag{14}$$

must hold. This result plays an important role in the theory of the kernels [3].

4. Let us define the linear operator in $\Sigma_1$

$$s_u^+(P) = E_1\{l(P, Q) + K_2(P, Q), u(Q)\}. \tag{15}$$

From the continuity properties of the kernels $l$ and $K_2$ one can easily show that this operator is completely continuous and, hence, treat the eigenvalue problem

$$u_\rho(P) = \kappa_\rho s_{u_\rho}^+(P), \qquad P \in D_1, \tag{16}$$

by the classical methods of Hilbert space theory. We obtain a discrete spectrum of eigenvalues $\kappa_\rho$ and corresponding eigenfunctions $u_\rho(P)$ which we may assume to form a complete orthonormal set in $\Sigma_1$. By the reasoning of the last section, one can easily prove

$$|\kappa_\rho| > 1. \tag{16'}$$

We have in $D_1$ the Fourier developments

$$K_1(P, Q) = \sum_{\rho=1}^{\infty} u_\rho(P)u_\rho(Q), \qquad l(P, Q) + K_2(P, Q) = \sum_{\rho=1}^{\infty} \frac{1}{\kappa_\rho} u_\rho(P)u_\rho(Q). \tag{17}$$

Using the definitions (3), we easily derive from (17):

$$G_2(P, Q) - G_1(P, Q) = \frac{1}{2} \sum_{\rho=1}^{\infty} \left(1 - \frac{1}{\kappa_\rho}\right) u_\rho(P)u_\rho(Q). \tag{18}$$

Consider the $\mathfrak{S}^+$-transforms of the eigenfunctions $u_\rho(P) \in \Sigma_1$ which are extended into functions of the class $\Sigma$ by defining them to be identically zero in $D_0$. Let

$$v_\rho(P) = \mathfrak{S}_{u_\rho}^+(P) = -2E_1\{G_2(P, Q), u_\rho(Q)\}, \qquad P \in D_0. \tag{19}$$

The $\{v_\rho(P)\}$ form an orthogonal set of functions in $\Sigma_0$; they satisfy

$$v_\rho(P) = -\kappa_\rho E_0\{l(P, Q) + K_2(P, Q), v_\rho(Q)\} \tag{20}$$

and thus play a very similar role in $\Sigma_0$ as did the $u_\rho$ in $\Sigma_1$. We calculate

$$E_0\{v_\rho, v_\sigma\} = \left(1 - \frac{1}{\kappa_\rho^2}\right) \delta_{\rho\sigma}. \tag{21}$$

All $v_\rho(P)$ vanish on $C_2$; they form a complete set of functions in the subspace of $\Sigma_0$ which consists of all elements vanishing on $C_2$. The function $G_2(P, Q) - G_0(P, Q)$ belongs to this subspace and can, therefore, be developed into a Fourier series in terms of the $v_\rho(P)$:

$$G_2(P, Q) - G_0(P, Q) = \frac{1}{2} \sum_{\rho=1}^{\infty} \frac{\kappa_\rho}{\kappa_\rho - 1} v_\rho(P)v_\rho(Q). \tag{22}$$

Since the $v_\rho(P)$ can be calculated from the $u_\rho(P)$ by elementary integra-

tion if $G_2$ is known, we see that the $u_\rho(P)$ permit a representation of the Green's functions of the complementary domains $D_0$ and $D_1$.

Using instead of $s^+$ the linear operator

$$s_n^-(P) = E_1\{l(P, Q) - K_2(P, Q), u(Q)\} \tag{23}$$

we can develop an analogous theory for Neumann's functions.

5. The function $w_\rho(P) \in \Sigma$ which has the value $\frac{1}{\kappa_\rho} u_\rho(P)$ in $D_1$ and $v_\rho(P)$ in $D_0$ is the $\mathfrak{S}^+$-transform of the function $u_\rho \in \Sigma$ which has been extended from $\Sigma_1$ by defining it identically zero in $D_0$. Using the known discontinuities of the function $u$ across $C_1$ we derive

$$v_\rho(P) = -\left(1 - \frac{1}{\kappa_\rho}\right) u_\rho(P), \qquad P \in C_1 \tag{24}$$

$$\frac{\partial v_\rho(P)}{\partial \nu} = \left(1 + \frac{1}{\kappa_\rho}\right) \frac{\partial u_\rho(P)}{\partial \nu}, \qquad P \in C_1 \tag{24'}$$

Thus, the boundary values and the values of the normal derivatives of the functions $v_\rho \in \Sigma_0$ and $u_\rho \in \Sigma_1$ are proportional along the common boundary $C_1$ of their domains of definition.

The function $\omega_\rho(P) \in \Sigma$ which is defined as $\left(\frac{1}{\kappa_\rho} - 1\right) u_\rho(P)$ in $D_1$ and as $v_\rho(P)$ in $D_0$ has the following property: It is continuous in $D_2$, vanishes on the boundary $C_2$ of $D_2$ and satisfies along the curve $C_1$ the discontinuity condition

$$\frac{\partial \omega_\rho}{\partial \nu^+} = -\frac{\kappa_\rho + 1}{\kappa_\rho - 1} \frac{\partial \omega_\rho}{\partial \nu^-}. \tag{25}$$

Thus, the eigenvalue $\kappa_\rho$ can also be characterized by an eigenvalue problem for discontinuous solutions of (1) of the Stekloff type [4].

* Work done under Navy Contract N5ori 76/XVI NR 043 046, at Harvard University.

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# GENERALIZED GROUP ALGEBRAS

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The purpose of this note is to describe a natural banach algebra associated with any topological group; the theory of this algebra and its subalgebras subsumes much of the standard $L^1$ theory, and is independent of the existence of a measure. Proofs will appear elsewhere.[1]

Let $G$ be an arbitrary topological group, not necessarily locally compact, and let $K$ be the complex field. Let $C$ be the banach space of all bounded *uniformly* continuous functions on $G$ to $K$, with norm $\|f\| = \sup_{x \in G} |f(x)|$. Let $B(C)$ be the banach algebra of all bounded linear operators on $C$. For $b \in G$, let $U_b$ be the left translation operator which sends a function $f$ into a function whose value at $x$ is $f(bx)$, and let $U^b$ be the corresponding right translation. By our choice of $C$, $U_b$ and $U^b$ are in $B(C)$ and have norm 1; the mapping $x \to U_x$ ($x \to U^x$) is an anti-isomorphism (isomorphism) of $G$ in $B(C)$, not in general continuous.

DEFINITION: *Let $\mathfrak{B}$ be the set of all $T$ in $B(C)$ which commute with all the operators $U_b$.*

$\mathfrak{B}$ is a closed subalgebra of $B(C)$; it is the centralizer in $B(C)$ of the subalgebra generated by the left representation of $G$. Since $U^x$ and $U_y$ commute, $\mathfrak{B}$ contains the right representation of $G$ in $B(C)$, and we may therefore regard $G$ as embedded in $\mathfrak{B}$. Consider $\hat{G}$, the set of all bounded continuous homomorphisms of $G$ into $K^\circ$, the multiplicative group of $K$. Clearly, $\hat{G}$ is a subset of the unit sphere in $C$.

THEOREM 1. *A function $f$ in $C$ is a character or a multiple of a character if and only if $f$ is simultaneously an eigenfunction for every operator in $\mathfrak{B}$.*

Thus, $\mathfrak{B}$ determines $\hat{G}$ and hence $G$, when $G$ is such that the duality theorem holds. Regarding $G$ as embedded in $\mathfrak{B}$, it has two topologies, the group topology carried over by the embedding, and the relative norm topology which it acquires as a subset of $\mathfrak{B}$; the latter is usually discrete.

THEOREM 2. *Any character of $G$ has an extension to a ring homomorphism of $\mathfrak{B}$ into $K$, continuous in the norm topology.*

If $h \in \hat{G}$, then the extension $H$ is defined by $H(T) = \lambda$, where $\lambda$ is the eigenvalue of $h$ under $T$. The kernel of $H$ is simply the set of all $T$ in $\mathfrak{B}$ with $T(h) = 0$, the origin of $C$.

COROLLARY: *$\hat{G}$ has a natural one-to-one embedding in $Hom(\mathfrak{B}:K)$ the set of all continuous ring homomorphisms of $\mathfrak{B}$ onto $K$.*

Clearly, any homomorphism $H$ of $\mathfrak{B}$ onto $K$ has a contraction to $G$ which is a group homomorphism of $G$ into $K^\circ$. (This remark, together with the preceding theorem, essentially contains the results obtained by Kakutani

and Kodaira.[2]) However, this homomorphism is not necessarily in $\check{G}$ since it need not be continuous in the group topology of $G$. Under certain conditions, this continuity can be forced. For example, this is the case if there is an operator $T_0 \in \mathfrak{B}$ such that $H(T_0) = 1$ while the map $x \to U^x T_0$ is continuous. This is the mechanism underlying the usual $L^1$ theory.

Since $\mathfrak{B}$ is large, the study of its subalgebras is of importance. Let $\mathfrak{A}_0$ be the closed subalgebra generated by the image of $G$ in its natural embedding. We call a subalgebra $\mathfrak{A}$ total if $T(f) = 0$ for every $T \in \mathfrak{A}$ implies that $f = 0$. It is convenient to single out the class of linear functionals $L$ on $\mathfrak{B}^+$ having the form $L(T) = T(\varphi)(e)$ where $\varphi \in C$ and $e$ is the neutral element of $G$.[3] We term such regular functionals; they form a subspace of the dual of $\mathfrak{B}^+$, usually not closed. The homomorphisms which arise as extensions of those in $G$ are regular. Given a subalgebra $\mathfrak{A} \subset \mathfrak{B}$, $\hat{G}$ has a natural one-to-one embedding in Hom($\mathfrak{A}$:$K$) which in certain cases is onto.

THEOREM 3. *Let $\mathfrak{A}$ be a closed total subalgebra of $\mathfrak{B}$ such that (1) $\mathfrak{A}_0\mathfrak{A} \subset \mathfrak{A}$ and $\mathfrak{A}\mathfrak{A}_0 \subset \mathfrak{A}$, (2) the map $x \to U^x T$ is continuous for each $T \in \mathfrak{A}$, (3) if $H$ is any homomorphism of $\mathfrak{A}$ onto $K$, then $H(U^b T) = H(TU^b)$ for all $b \in G$ and $T \in \mathfrak{A}$, (4) every member of Hom($\mathfrak{A}$:$K$) is the limit of regular functionals. Then, every homomorphism of $\mathfrak{A}$ onto $K$ arises as the unique extension of a member of $\hat{G}$.*

To show the connection of the preceding with the usual theory, let $\mathfrak{L}$ be the dual space of $C$. The correspondence $T \to F$, where $F(f) = T(f)(e)$ may be shown to be a norm preserving isomorphism of the banach spaces $\mathfrak{B}$ and $\mathfrak{L}$. Since $\mathfrak{B}$ has the additional structure of an algebra, $\mathfrak{L}$ can be given the same structure. Denoting the resulting multiplication for functionals by $*$, $F_1 * F_2$ turns out to be the customary convolution of the functionals $F_1$ and $F_2$, in the sense of Radon measures.[4] The operator $U^b$ in $\mathfrak{B}$ corresponds to the point functional $\theta_b$ where $\theta_b(f) = f(b)$. The theory of the Fourier transform assumes the following simple form: given $F \in \mathfrak{L}$, let $\hat{F}$ be its restriction to the subset $\hat{G} \subset C$. The importance of discussing the span of $\hat{G}$ in $C$ is evident. For the usual $L^1$ theory, choose $G$ to be locally compact abelian, and let $\mathfrak{A}$ be the set of all operators of the form $g(x) = \int f(xy)\varphi(y)\,dy$ where $\varphi \in L^1(G)$ and $f \in C$. The hypotheses of Theorem 3 are easily verified.

The norm topology on $C$ is not the only usable one; in particular, that of uniform convergence on compact sets deserves special consideration. The results for this case are similar, and will appear elsewhere.

[1] There seems to be some relation between the present approach and that in a recent abstract of Hewitt and Zuckerman, *Bull. Am. Math. Soc.*, **55**, 1057 (1949). See also I. E. Segal, *Ann. Math.*, **51**, 293–298 (1950). The two-sided regular representation of a unimodular locally compact group.

[2] Kakutani and Kodaira. Normed ring of a locally compact abelian group, *Proc. Imp. Acad. (Tokyo)*, **19**, 360–365 (1943).

[3] $B^+$ is the underlying banach space of the algebra $B$.

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# LIMIT CYCLES OF SYSTEMS OF THE SECOND ORDER

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The present paper deals with a number of questions about the limit cycles of systems

$$\dot{x} = P(x, y), \qquad \dot{y} = Q(x, y). \tag{1}$$

The importance in non-linear mechanics of these limit cycles, or isolated periodic solutions, is well known. It has led to a variety of methods for finding these solutions which yield some information in noteworthy special cases. In Princeton lectures in the fall of 1949, Lefschetz introduced and applied to certain questions the following method: a rotation by a fixed angle $\alpha$ being applied to the vector field $(X, Y)$ in the phase plane of the system (1) gave rise to a new vector field $(X \cos \alpha - Y \sin \alpha, X \sin \alpha + Y \cos \alpha)$, whose trajectories were then compared to those of the original system. A method essentially like that of Lefschetz is utilized in this note, and has made it possible to answer systematically many questions concerning limit cycles of second order systems.

Consider then a family of systems

$$\dot{x} = P(x, y, \alpha), \qquad \dot{y} = Q(x, y, \alpha). \tag{2}$$

We shall say that these equations form a complete family, and that the associated vector fields in the $(x, y)$ plane form a complete family of rotated vector fields in the plane, if the following conditions are satisfied:

(*a*) $P$ and $Q$ satisfy Lipschitz conditions with respect to $x$ and $y$, and are differentiable functions of $\alpha$.

(*b*) The zeros of $P^2 + Q^2$ remain fixed in the $(x, y)$ plane as $\alpha$ varies.

(*c*) The condition

$$P\frac{\partial Q}{\partial \alpha} - Q\frac{\partial P}{\partial \alpha} > 0$$

is satisfied at all points where $P_2 + Q^2 > 0$.

(*d*) $P$ and $Q$ are periodic functions of $\alpha$ with minimum period $2\pi$, and furthermore

$$P(x, y, \alpha + \pi) = -P(x, y, \alpha),$$
$$Q(x, y, \alpha + \pi) = -Q(x, y, \alpha).$$

As $\alpha$ varies, one may picture the field vectors as turning, at a positive rate, all in the same sense, and in such a way that, in the interval $0 \leq \alpha < \pi$, all have turned through the angle $\pi$. Thus the original field, but with the vectors reversed, appears again. An example is the set of uniform rotations of the field vectors by an angle $\alpha$. The critical points remain fixed, according to condition ($b$). We shall study the phase portraits of these fields, and will show that they are closely related.

We note that the index (in the sense of Poincaré) of any closed curve in the plane, with respect to any field of a complete family, is independent of $\alpha$. It may also be verified that if a change of the dependent variables of the form $x_1 = x_1(x, y)$, $y_1 = y_1(x, y)$, with functional determinant everywhere different from zero, is made, then the differential equations in $x_1$ and $y_1$ also constitute a complete family.

The following theorems give fundamental properties of complete families as defined above:

THEOREM I. *Limit cycles corresponding to different values of $\alpha$ do not intersect.*

THEOREM II. *Limit cycles which are completely stable or completely unstable expand or contract monotonely as $\alpha$ varies in a fixed sense. Semistable cycles either split into two diverging cycles or disappear.*

THEOREM III. *Each elementary critical point of positive determinant $\Delta$ either generates or absorbs exactly one cycle in the range $0 \leq \alpha < \pi$.*

We sketch a proof of Theorem III for the case in which the rotations are uniform over the plane. Let

$$\dot{x} = X \cos \alpha - Y \sin \alpha,$$
$$\dot{y} = X \sin \alpha + Y \cos \alpha,$$

be the equations of the family, where

$$X = ax + by + X_2,$$
$$Y = cx + dy + Y_2,$$

and $X_2$ and $Y_2$ contain only terms of the second and higher orders in $x$ and $y$. The characteristic roots of the linear equations of first approximation near the origin are given by

$$\lambda^2 - [(a + d) \cos \alpha + (b - c) \sin \alpha]\lambda + ad - bc = 0.$$

Writing

$$\Delta = ad - bc,$$

$$\Gamma^2 = (a + d)^2 + (b - c)^2,$$

and

$$\tan \delta = \frac{a + d}{c - b},$$

we can write the characteristic roots in the form

$$\lambda = +\frac{\Gamma}{2}\sin(\alpha - \delta) \pm \frac{\sqrt{\Gamma^2 \sin^2(\alpha - \delta) - 4\Delta}}{2}.$$

If $\Delta > 0$, corresponding to an index of $+1$, these roots assume both real and complex values, since $4\Delta \leq \Gamma^2$. For $\alpha = \delta$, the roots are purely imaginary, and the origin is a center. For nearby values of $\alpha$, the origin is a focus, or spiral point, with the stability of the spirals changing sense when $\alpha = \delta$. According to Theorem I, the generation of such a cycle can take place only once in a half revolution of the field.

With the aid of these theorems, it can be shown that regions of certain types in the phase plane are swept out by the cycles of a given complete family. Examples of such regions are: the interior of a given cycle containing a single critical point, which is elementary, or an annulus free of critical points which is bounded by two cycles of the family not necessarily belonging to the same value of $\alpha$. In some cases the whole plane is covered. These results, together with Theorem I, lead to criteria for the non-existence in such regions of limit cycles of other equations of the given complete family.

The theorems quoted above also suggest the following method of locating limit cycles. Embed the given equation in a complete family of rotations and calculate the values of $\delta$ for the elementary critical points of index $+1$. The expansion of a cycle generated at one of these points may be calculated as follows: When the cycle is very small, the linear terms in the expansions of Theorem III predominate in the right-hand sides of the equations and it is possible to use Poincaré's method of generating cycles to determine the size. This method picks out the closed solution of an equation with small non-linear terms from among the closed solutions of the corresponding linear equation. The rate of expansion may subsequently be found with the following formula for $\frac{dn}{d\alpha}$ the rate of expansion of the cycle along its normal: Let $T$ be the period and $h$ the exponent of the cycle. Let $v(t)$ denote the "velocity" $(P^2 + Q^2)^{1/2}$. Then,

$$\frac{dn(t)}{d\alpha} = \frac{\oint \exp\left[-\int_t^{t+\tau}(P_x + Q_y)d\tau'\right]\left\{P\frac{\partial Q}{\partial \alpha} - Q\frac{\partial P}{\partial \alpha}\right\}_{t+\tau} d\tau}{v(t)\,[1 - \exp(-hT)]}.$$

The cycle may disappear in one of the following ways: (*a*) by coalescing with an exterior or interior cycle of opposite stability (in this case, reverse the variation of $\alpha$ and follow the other cycle); (*b*) by tending to a saddle point or other critical point; (*c*) by tending to infinity in one or more directions.

If the cycle in question fails to persist until the value of $\alpha$ corresponding to the given equations is reached, then Theorem I asserts that no part of any cycle of this system lies in the region swept out during the expansion. For any value of $\alpha$ the cycle may become a band of positive width of closed cycles. When $\alpha$ has run through an interval of length $\pi$, each cycle must either have disappeared or else have taken the place of another cycle of the original field. Thus the method will always find those limit cycles which enclose one critical point with $\Delta > 0$, and it provides a criterion of non-existence, in case there are no such cycles.

In more complicated situations where the cycle might surround several critical points one must establish the existence of a cycle of the family for some value of $\alpha$. This may be accomplished by finding a closed curve without contact with the given field (a Bendixson curve), and then choosing the complete family in such a way that this curve becomes a limit cycle for a particular value of $\alpha$.

THEOREM IV. *All cycles of a given field containing the same critical points as a given Bendixson curve can be constructed by rotation operations of a suitable complete family.*

I wish to thank Professors S. Lefschetz and M. Schiffer for their interest and advice concerning this work.

## ON THE ADDITION AND MULTIPLICATION THEOREMS FOR SPECIAL FUNCTIONS*

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Some years ago I called attention to the unification in the theory of special functions achieved through systematic exploitation of properties of the solutions of the $F$-equation[1]

$$\frac{\partial F(z, \alpha)}{\partial z} = F(z, \alpha + 1). \tag{1}$$

One virtue of the method is that many formal relationships are discovered and demonstrated in an automatic and trivial fashion, relationships which, if previously known at all, were originally derived at greater length and with more elaborate analytical tools. In particular, if $F(z + y, \alpha)$ be an analytic function of $y$, we have by Taylor's theorem the addition formula

$$F(z + y, \alpha) = \sum_{n=0}^{\infty} \frac{y^n}{n!} F(z, \alpha + n), \tag{2}$$

which contains as special cases most of the addition formulae and many of the generating expansions for familiar special functions.[3] In this note I wish to point out that this same expansion may be written as a multiplication theorem. First we put $y - z$ for $y$ in (2):

$$F(y, \alpha) = \sum_{n=0}^{\infty} \frac{(y - z)^n}{n!} F(z, \alpha + n). \tag{3}$$

If $y = kz$ then (3) becomes the multiplication theorem

$$F(kz, \alpha) = \sum_{n=0}^{\infty} \frac{(k - 1)^n z^n}{n!} F(z, \alpha + n), \tag{4}$$

which is the desired result.

If, in the general multiplication theorem (4), we put

$$F(z, \alpha) = e^{i\alpha\pi} z^{-\alpha/2} J_\alpha (2\sqrt{z}), \tag{5}$$

where $J_\alpha(x)$ is the Bessel function of the first kind, after some reductions we readily obtain the known result[3]

$$J_\alpha(kz) = k^\alpha \sum_{n=0}^{\infty} \frac{(1 - k^2)^n}{n!} \left(\frac{z}{2}\right)^n J_{\alpha+n}(z). \tag{6}$$

If $J_\alpha$ be replaced by $Y_\alpha$, (6) remains valid. If instead we put

$$F(z, \alpha) = \Gamma(\alpha + 1)\, (-z)^{-\alpha-1-b} e^{-\frac{1}{z}} L_\alpha^{(b)} (1/z) \tag{7}$$

where $L_\alpha^{(b)}(z)$ is the generalized Laguerre function, by substitution in (5) follows the first multiplication theorem of Erdélyi:[4]

$$k^{\alpha+1+b} e^{-kz} L_\alpha^{(b)} (kz) = \sum_{n=0}^{\infty} \binom{\alpha+n}{n} \left(1 - \frac{1}{k}\right)^n e^{-z} L_{\alpha+n}^{(b)} (z), \tag{8}$$

while if we put

$$F(z, \alpha) = e^{i\alpha\pi} e^{-z} L_b^{(\alpha)} (z), \tag{9}$$

we similarly obtain the second multiplication theorem of Erdélyi:[5]

$$e^{-kz} L_b^{(\alpha)} (kz) = \sum_{n=0}^{\infty} \frac{(1 - k)^n z^n}{n!} e^{-z} L_b^{(\alpha+n)} (z). \tag{10}$$

Of the at least eleven multiplication theorems which hold for the hypergeometric functions, it may be of interest to write down two of the simplest, those resulting from the respective choices

$$F(z, \alpha) = e^{i\alpha\pi} \Gamma(\alpha) z^{-\alpha} F\left(\alpha, c; b; \frac{1}{z}\right), \tag{11}$$

$$F(z, \alpha) = \frac{\Gamma(\alpha - b)\,\Gamma(\alpha - c)}{\Gamma(\alpha)}(1 - z)^{b+c-\alpha}F(b, c;\ \alpha;\ z), \quad (12)$$

namely,

$$F(a, b;\ c;\ kz) = k^{-a}\sum_{n=0}^{\infty}\binom{a+n-1}{n}\left(1 - \frac{1}{k}\right)^n F(a + n, b;\ c;\ z). \quad (13)$$

$$F(a, b;\ c;\ kz) = \left(\frac{1 - z}{1 - kz}\right)^{a+b-c}\sum_{n=0}^{\infty}\left(\frac{k - 1}{1 - \frac{1}{z}}\right)^n\binom{1-c+a}{n}\binom{1-c+b}{n}\binom{n}{1-c}$$
$$F(a, b;\ c + n;\ z). \quad (14)$$

Finally, we may put

$$F(z, \alpha) = e^{i\alpha\pi}\,\Gamma(\alpha)\zeta(\alpha, z), \quad (15)$$

where $\zeta(\alpha, z)$ is the generalized Euler-Riemann zeta function, thus obtaining the expansion

$$\zeta(a, kz) = \sum_{n=0}^{\infty}\binom{a+n-1}{n}(1 - k)^n z^n\,\zeta(a + n, z), \quad (16)$$

a result which when $z = 1$ yields a formula for $\zeta(\alpha, k)$ as a series of simple zeta functions.

We conclude with three remarks. The first is that since all the results of this note have been derived by simple substitutions in (2), without use of any limit operation, and since (2) is valid whenever the series on the right converges, it follows that all our results are valid subject only to convergence of the series involved. The second is that since $k$ and $z$ occur symmetrically on the left side of (4), by interchanging them upon the right side and equating the results we obtain the reciprocal theorem

$$\sum_{n=0}^{\infty}\frac{(k - 1)^n z^n}{n!}F(z, a + n) = \sum_{n=0}^{\infty}\frac{(z - 1)^n k^n}{n!}F(k, \alpha + n), \quad (17)$$

subject to the convergence of both series. The third is that by putting $k = 0$ the formula (4) enables us to find

$$\phi(\alpha) = F(0, \alpha) = \sum_{n=0}^{\infty}\frac{(-)^n z^n}{n!}F(z, \alpha + n), \quad (18)$$

a result which is interesting both because the left-hand side is independent of $z$ and because the function $\phi(\alpha)$ occurs explicitly in many of the theorems concerning solutions of the $F$-equation.

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[1] Truesdell, C., *An Essay Toward a Unified Theory of Special Functions*, Princeton, 1948.

[2] Op. cit., ¶14.

[3] Watson, J. N., *A Treatise on the Theory of Bessel Functions*, Cambridge, 1922. See ¶5.22.

[4] Erdélyi, A., "Funktionalrelationen mit konfluenten hypergeometrischen Funktionen," I, *Math. Z.*, **42**, 125–143 (1936–1937), eq. (5,3).

[5] Op. cit., eq. (6,2). In this note we employ the definition of the generalized Laguerre function given by E. Pinney, "Laguerre Functions in the Mathematical Foundations of the Electromagnetic Theory of the Paraboloidal Reflector," *J. Math. Phys.*, **25**, 49–79 (1946), so that the lower index is not restricted to integer values. Thus our results (8) and (10) are fully equivalent to Erdélyi's formulae (5,1) and (6,1), which are expressed in terms of the $M_{k,m}$ functions of Whittaker. Generalizations of Erdélyi's formulae (5,4) and (6,3) may be obtained either by applying Kummer's first transformation to our expansions (8) and (10) or by inserting successively the two solutions $z^{-a}L_b^{(-a)}(z)/\Gamma(b-a+1)$ and $\Gamma(a-b)\,(-z)^{-a}L_{-a}^{(b)}\left(\frac{1}{z}\right)$ into the general multiplication theorem (4).

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